ICGEB Capacity-Building Project in the Republic of Cameroon

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Lab Expert, National Public Health Emergencies Operations Coordination Centre

“To enhance detection capacity to respond to SARS-CoV-2 and other emerging infections in Sub-Saharan Africa by assessing and transferring cost-effective technology for rapid viral identification and surveillance”
OUTLINE

I. CAMEROON IN A NUTSHELL

II. INFECTIOUS DISEASE BURDEN

III. ICGEB CONTRIBUTION IN DIAGNOSTICS

IV. ICGEB CONTRIBUTION IN GENOMIC SURVEILLANCE

V. PANDEMIC PREPAREDNESS WITH ICGEB

VI. TAKE HOME MESSAGE
I. CAMEROON IN A NUTSHELL

Location: A country at the Centre of Africa

Capital cities: Yaoundé (political) & Douala (economical)

Overall surface: 466,050 km²

Population: 28.6 million in 2023 (2.63% annual increase)

Official languages: French and English
### II. INFECTIOUS DISEASE BURDEN (1/4)

<table>
<thead>
<tr>
<th>Disease surveillance week 48</th>
<th>New Cases</th>
<th>New Deaths</th>
<th>Cumul Cases</th>
<th>Cumul Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthrax (&quot;charbon&quot; in french)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chikungunya</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholera</td>
<td>47</td>
<td>3</td>
<td>4238</td>
<td>153</td>
</tr>
<tr>
<td>Dengue</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dracunculiasis</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral haemorrhagic fever (last was Lyell syndrome)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Yellow fever</td>
<td>35</td>
<td>0</td>
<td>1322</td>
<td>17</td>
</tr>
<tr>
<td>Typhoid fever (all 10 regions)</td>
<td>13376</td>
<td>20</td>
<td>609307</td>
<td>472</td>
</tr>
<tr>
<td>Meningitis</td>
<td>13</td>
<td>1</td>
<td>1057</td>
<td>46</td>
</tr>
<tr>
<td>Malaria (all 10 regions)</td>
<td>45 333</td>
<td>86</td>
<td>2099920</td>
<td>2673</td>
</tr>
<tr>
<td>Poliomyelitis</td>
<td>7</td>
<td>0</td>
<td>633</td>
<td>11</td>
</tr>
</tbody>
</table>
## II. INFECTIOUS DISEASE BURDEN (2/4)

<table>
<thead>
<tr>
<th>Disease surveillance week 48</th>
<th>New Cases</th>
<th>New Deaths</th>
<th>Cumul Cases</th>
<th>Cumul Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plague (&quot;Peste&quot; in french)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dog bites</td>
<td>150</td>
<td>0</td>
<td>5994</td>
<td>19</td>
</tr>
<tr>
<td>Snake bites</td>
<td>160</td>
<td>15</td>
<td>8873</td>
<td>103</td>
</tr>
<tr>
<td>Rabies</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Measles</td>
<td>16</td>
<td>0</td>
<td>9135</td>
<td>57</td>
</tr>
<tr>
<td>SRAS</td>
<td>3</td>
<td>0</td>
<td>590</td>
<td>22</td>
</tr>
<tr>
<td>Flu syndrome</td>
<td>4934</td>
<td>7</td>
<td>215951</td>
<td>102</td>
</tr>
<tr>
<td>Mpox</td>
<td>0</td>
<td>0</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>Smallpox</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Diphtheria</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>COVID-19</td>
<td>1</td>
<td>0</td>
<td>808</td>
<td>11</td>
</tr>
</tbody>
</table>
Dynamics of COVID-19 burden in Cameroon: 2020 – 2022

**Legend:**
- Order of wave
- Duration in weeks
- Period (in weeks), Year
- Date start – Date end
- Nber confirmed cases
- Nber hospitalised
- Nber deaths
- Case Fatality rate
- Viral strains isolated by sequencing (%)

**First wave**
- 16 weeks
- 27/04/2020 - 16/08/2020
- 16,948 confirmed cases
- 1,847 hospitalised
- 386 deaths
- 2.3% Case Fatality rate
- Viruses of the lineage of origin (100%)

**Second wave**
- 21 weeks
- 1/11/2020 - 06/06/2021
- 52,271 confirmed cases
- 4,675 hospitalised
- 835 deaths
- 1.6% Case Fatality rate
- Non-variants of concern (70%)
- Alpha variant (20%)
- Beta variant (10%)

**Third wave**
- 11 weeks
- 15/06/2021 - 21/11/2021
- 21,753 confirmed cases
- 2,230 hospitalised
- 426 deaths
- 2.0% Case Fatality rate
- Non-variants of concern (60%)
- Delta variant (40%)

**Fourth wave**
- 8 weeks
- 06/09/2022 to 06/02/2022
- 10,803 confirmed cases
- 809 hospitalised
- 79 deaths
- 0.73% Case Fatality rate
- Omicron variant (90%)
- Non-variants of concern (10%)
COVID-19 under control in Cameroon since mid-2023 (Jan-Dec)
### National Laboratory capacity in response to COVID-19

<table>
<thead>
<tr>
<th>Nº</th>
<th>Key indicators</th>
<th>March 2020, (n)</th>
<th>March 2022 (n)</th>
<th>Specific comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.</td>
<td>Number of frameworks for the genomic surveillance platform</td>
<td>0</td>
<td>1</td>
<td>Strong governmental engagement (ministerial decision, April 12, 2021)</td>
</tr>
<tr>
<td>ii.</td>
<td>Number of national strategies for SARS-CoV-2 genomic surveillance</td>
<td>0</td>
<td>2</td>
<td>The first plan has been revised as per changes in the pandemic.</td>
</tr>
<tr>
<td>iii.</td>
<td>Number of laboratories with the capacity for COVID-19 molecular testing</td>
<td>1</td>
<td>45</td>
<td>24 public laboratories and 21 private laboratories</td>
</tr>
<tr>
<td>iv.</td>
<td>Number of laboratories with the capacity for variant screening by PCR point mutation assay</td>
<td>0</td>
<td>16</td>
<td>These are laboratories with open real-time PCR systems for SARS-CoV-2</td>
</tr>
<tr>
<td>v.</td>
<td>Number of laboratories with the capacity/network for SARS-CoV-2 sequencing</td>
<td>0</td>
<td>6</td>
<td>5 public labs and 1 private lab (performing targeted and/or whole-genome sequencing)</td>
</tr>
<tr>
<td>vi.</td>
<td>PCR-positive samples successfully processed for SARS-CoV-2 genomic surveillance</td>
<td>0</td>
<td>3,881</td>
<td>1,509 PCR-mutation assays, 1,612 targeted sequencing, 760 whole-genome sequencing</td>
</tr>
</tbody>
</table>
First experience with the RT-LAMP technology for COVID-19

Loop-Mediated Isothermal Amplification:

- Loop-mediated isothermal amplification (LAMP) uses 4-6 primers recognizing 6-8 distinct regions of target DNA for a highly specific amplification reaction.

- A strand-displacing DNA polymerase initiates synthesis and 2 specially designed primers form “loop” structures to facilitate subsequent rounds of amplification through extension on the loops and additional annealing of primers.
III. ICGEB CONTRIBUTION IN DIAGNOSTICS (3/6)

First experience with the RT-LAMP technology for COVID-19

First step: Viral RNA isolation using a standard commercial kit (QIAamp® Viral RNA Mini Kit, Qiagen).

Second step: Amplification using a thermocycler at a single temperature of 65°C.
Results of COVID-19 RT-LAMP on nasopharyngeal swabs

Acceptable performance of RT-LAMP on nasopharyngeal specimens
(excellent outcome with high viral loads)

<table>
<thead>
<tr>
<th>Stratification</th>
<th>N</th>
<th>Sensitivity % (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT&lt;25</td>
<td>129</td>
<td>98 (93-100)</td>
</tr>
<tr>
<td>CT≥25</td>
<td>93</td>
<td>63 (53-73)</td>
</tr>
</tbody>
</table>

Diagnostic performance of a colorimetric RT-LAMP for the identification of SARS-CoV-2: A multicenter prospective clinical evaluation in sub-Saharan Africa


Research Paper

Diagnostic performance of a colorimetric RT-LAMP for the identification of SARS-CoV-2: A multicenter prospective clinical evaluation in sub-Saharan Africa

III. ICGEB CONTRIBUTION IN DIAGNOSTICS (5/6)

Results of COVID-19 RT-LAMP on saliva without extraction

Overall acceptable performance of RT-LAMP on saliva (n= 970)

CT< 37 (National threshold)

<table>
<thead>
<tr>
<th>RT PCR (REFERENCE: Gold standard)</th>
<th>+</th>
<th>-</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT-LAMP (Under evaluation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>44</td>
<td>33</td>
<td>77</td>
</tr>
<tr>
<td>-</td>
<td>11</td>
<td>798</td>
<td>809</td>
</tr>
<tr>
<td>TOTAL</td>
<td>55</td>
<td>831</td>
<td>886</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>80.0%</td>
<td>68.4% to 88.6%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.0%</td>
<td>95.3% to 96.6%</td>
</tr>
<tr>
<td>Positive Predictive Value (*)</td>
<td>57.1%</td>
<td>48.8% to 63.3%</td>
</tr>
<tr>
<td>Negative Predictive Value (*)</td>
<td>98.6%</td>
<td>97.9% to 99.2%</td>
</tr>
</tbody>
</table>

Kappa= 0.64 CI : 0.54 to 0.74
III. ICGEB CONTRIBUTION IN DIAGNOSTICS (6/6)

Capacity-building on RT-LAMP for other laboratories

- Successful implementation in all labs: thermocycler, heating block, or water bath
- Result performance: 100% concordance trained lab versus reference lab (CIRCB)
Genomic surveillance of SARS-CoV-2 reveals highest severity and mortality of delta over other variants: evidence from Cameroon. Fokam et al., Nat Sc. Reports 2023

With contribution from ICGEB for whole genome sequencing (Bill & Melinda Gates sponsorship)
IV. ICGEB CONTRIBUTION IN GENOMIC SURVEILLANCE IN CAMEROON (2/5)

Targeted sequencing of SARS-CoV-2 of positive samples

Sanger-sequencing and interpreted of variants using Stanford db.v9.5

- **Site:** Virology Laboratory of the “Chantal BIYA” International Reference Centre (CIRCB), Yaoundé, Cameroon

- **Laboratory technique:** design of Sanger sequencing of the spike region of SARS-CoV-2 positive specimens.

- **Training:** Laboratory sequencing testing with reference to ICGEB whole genome sequences.

Figure: Sanger sequencing pipeline
IV. ICGEB CONTRIBUTIUTION IN GENOMIC SURVEILLANCE IN CAMEROON (3/5)

Real-time sequencing of sub-variants in 2023

- **Current trend of COVID-19**: New Omicron sub-variants and recombinants with mild symptoms.

- **Genomic surveillance**: Atypical recombinants (BA.4.6/XBB.1), timely detect and track novel strains, related disease severity and risk of transmission for optimal pandemic control.

Figure: Phylogenetic tree of SARS-CoV-2 sequences obtained
IV. ICGEB CONTRIBUTION IN GENOMIC SURVEILLANCE IN CAMEROON (4/5)

WORKSHOP ONT SEQUENCING FOR SARS-CoV-2 in ITALY & CAMEROON

PORTABLE SEQUENCING DEVICE
- CE MARKED: YES
- Dimensions Size: W 105 mm, H 23 mm, D 33 mm
- Weight: 87 g

ICGEB-Italy

CIRCB-Cameroon

Fluorometer, benchtop
Qubit™ 4 Fluorometer, with WiFi

SuperScript™ IV First-Strand Synthesis System
Catalog number: 18091050

R9 Flowcells (Oxford Nanopore)
R9 Flowcells (Oxford Nanopore)
Flow Cells for MiniION and MiniION Mk1C
Supplier: Oxford Nanopore Technologies
Nanopore sequencing flow cells for use in sequencing

Incubators, laboratory, thermocycler
qTOWER³ G touch
qTOWER³ G touch (230 V), incl. color module 1

PC workstation
ESPRIMO P5011
Personal computer with licensed operating system

Primers: SARS-CoV-2 (ref. Diatheva)
5 kit for 100 reactions

Primers: SARS-CoV-2 (ref. Diatheva)
IV. ICGEB CONTRIBUTION IN GENOMIC SURVEILLANCE IN CAMEROON (5/5)

SETTING-UP OF A BIOINFORMATICS UNIT

- Visit Minister of Health & Diplomates
- Dr NKA, Head Unit trained on Bioinformatics at CERI – Prof Tulio

- **Bioinformatics unit**: Office workspace provided to the team;
- **Staff**: Head of unit & staff designated in the Virology Laboratory;
- **Basic equipment**: High throughput computers already in place;
- **Staff training**: further opportunities identified (ICGEB, CERI, Africa CDC);
- **pending needs**: a server/cluster for sequence data storage & sharing;
- **Vision**: become a bioinfo Ref. centre for surveillance in Central Africa.
V. PANDEMIC PREPAREDNESS WITH ICGEB (1/3)

National workshop to scale-up RT-LAMP in district laboratories of Cameroon for the surveillance of emerging pathogens

RT-LAMP laboratory training

RT-LAMP Result interpretation

Collaboration with the National Public Health Lab & the Public Health Emergencies Centre
Rollout of RT-LAMP in 10 Laboratories of Cameroon for community-based surveillance of emerging pathogens

Established RT-LAMP Lab network:

1. **CIRCB**: Lead reference lab.
2. **NPHL**: Yaoundé urban;
3. **Ekoumdoum district hosp**: Yaoundé rural;
4. **Dang district hosp**: Northern region;
5. **Olamze district hosp**: Southern boarder;
6. **EUC laboratory**: urban western region;
7. **DREAM laboratory**: rural western region;
8. **FHS laboratory**: urban southwest region;
9. **Limbe Hosp**: southwest boarder region;
10. **Abong-Mbang district hosp**: East region.

Note: Health facilities trained on RT-LAMP
Rollout of RT-LAMP in 10 African countries and in community laboratories for front-line surveillance of emerging pathogens

- The Project started officially on 20 August 2021 in five (05) African countries with nasopharyngeal swabs;
- Project expanded to 10 African countries with saliva testing and extraction-free;
- Project extended at the level of district laboratories for community-based surveillance of pathogens;
- Project network established for both epidemiological and genomic surveillance of other emerging pathogens (arboviral diseases) in sub-Saharan Africa.
VI. TAKE HOME MESSAGE

ICGEB Capacity-building in Cameroon:

- **RT-LAMP technology**: user-friendly across laboratories;

- **RT-LAMP implementation**: successful both with nasopharyngeal swabs and with saliva samples in across laboratories;

- **Genomic surveillance**: effective with sequencing of variants;

- **Extension of RT-LAMP technology to district laboratories**: an added-value for disease surveillance within the local communities;

- **Established network**: an effective system contributing for optimal pandemic preparedness and surveillance in sub-Saharan Africa.
THANK YOU

COVID-19 team (at start)

COVID-19 team (with ICGEB)

3rd INTERNATIONAL CONFERENCE ON PUBLIC HEALTH IN AFRICA
BREAKING BARRIERS POSITIONING AFRICA IN THE GLOBAL HEALTH ARCHITECTURE

BILL & MELINDA GATES FOUNDATION