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De nouveaux coronavirus de chauve-souris similaires à SARS-CoV-2 éclairent l'origine de la pandémie de COVID-19.

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+ corresponding author
Context

- Numerous SARS-CoV-2-related viruses identified in bats & pangolins
- First cases detected in Wuhan (Huanan seafood market & elsewhere)
- First case 8th December 2019

- Animal reservoir?
- Intermediate host?
- Routes of introduction into human populations?
- Silent circulation in humans before detection?
Objectives

• To improve the understanding of the **origins, natural history** and **dissemination** of SARS-CoV-2 and SARS-like coronaviruses in their **natural environment**
  - Identify natural reservoir(s) of SARS-CoV-2
  - Decipher interspecies transmission (bat-to-bat, bat-to-mammals, bat-to-humans)

• To detect **past coronavirus infections** in humans exposed to animal reservoirs
  - Identify early circulation of SARS-CoV-2 or SARS-CoV-2-like viruses before global spread
Workflow & consortium

Characterization of the virus

Ecological studies
- Hosts spectrum
- Seasonality
- Persistence in environment
- Virus tropism in bats
- etc.

Full genome sequencing
- Evolution history

Structural modelling
- Interaction with receptor

Pseudotyped lentiviruses
- Virus entry

Virus isolation
- In vivo studies

Expression structural proteins
- Seroprevalence studies

Ongoing studies

IPP-Laos
IPP-Pathogen discovery
IPP-Structural virology
IPP-Structural bioinformatics
IPP-Molecular Genetics of RNA Viruses
IPP-Mouse genetics
Field work

- **645 bats**
  - 48 species
  - 6 families

- **Sample types**
  - Oral swabs (608)
  - Rectal swabs (539)
  - Urine (157)
  - Blood (246)
pan-CoV screening

<table>
<thead>
<tr>
<th>Anal Swab ID</th>
<th>Bat Genus</th>
<th>Bat Species</th>
<th>CoV Genus</th>
<th>CoV Subgenus</th>
<th>Closest sequence</th>
<th>% blastN similarity</th>
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<tbody>
<tr>
<td>BANAL-20-27</td>
<td>Rhinolophus</td>
<td>pusillus</td>
<td>co-infection</td>
<td>Alpha/Betacoronavirus</td>
<td>unclassified</td>
<td>Rhinolophus bat coronavirus HKU32 / SARS-CoV-2</td>
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<tr>
<td>BANAL-20-51</td>
<td>Hipposideros</td>
<td>pomona</td>
<td>Alphacoronavirus</td>
<td>Decacovirus</td>
<td>Bat coronavirus HKU10</td>
<td>98%</td>
</tr>
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<td>BANAL-20-52</td>
<td>Rhinolophus</td>
<td>malayanus</td>
<td>Betacoronavirus</td>
<td>Sarbecovirus</td>
<td>SARS-CoV-2</td>
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<td>Rhinolophus</td>
<td>pusillus</td>
<td>Betacoronavirus</td>
<td>Sarbecovirus</td>
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<td>99%</td>
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<td>Betacoronavirus</td>
<td>Sarbecovirus</td>
<td>SARS-CoV-2</td>
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<td>98%</td>
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<td>Chaerephon</td>
<td>plicatus</td>
<td>Alphacoronavirus</td>
<td>Pedacovirus</td>
<td>Porcine epidemic diarrhea virus (PEDV)</td>
<td>93%</td>
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<td>plicatus</td>
<td>Alphacoronavirus</td>
<td>Pedacovirus</td>
<td>Porcine epidemic diarrhea virus (PEDV)</td>
<td>93%</td>
</tr>
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<td>Alphacoronavirus</td>
<td>Pedacovirus</td>
<td>Porcine epidemic diarrhea virus (PEDV)</td>
<td>93%</td>
</tr>
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<td>plicatus</td>
<td>Alphacoronavirus</td>
<td>Pedacovirus</td>
<td>Porcine epidemic diarrhea virus (PEDV)</td>
<td>93%</td>
</tr>
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<td>BANAL-20-236</td>
<td>Rhinolophus</td>
<td>marshalli</td>
<td>Betacoronavirus</td>
<td>Sarbecovirus</td>
<td>SARS-CoV-2</td>
<td>99%</td>
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<td>SARS-CoV-2</td>
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<td>Rhinolophus</td>
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<td>Betacoronavirus</td>
<td>Sarbecovirus</td>
<td>SARS-CoV-2</td>
<td>99%</td>
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<td>Hipposideros</td>
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<td>Alphacoronavirus</td>
<td>Decacovirus</td>
<td>Bat coronavirus HKU10</td>
<td>97%</td>
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<td>BANAL-20-273</td>
<td>Rhinolophus</td>
<td>pusillus</td>
<td>Alphacoronavirus</td>
<td>unclassified</td>
<td>Rhinolophus bat coronavirus HKU32</td>
<td>92%</td>
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<td>affinis</td>
<td>Alphacoronavirus</td>
<td>Rhinacovirus</td>
<td>Rhinolopbus affinis bat coronavirus HKU2-related / Swine acute diarrhea syndrome related coronavirus (SADS-CoV)</td>
<td>98%</td>
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<td>BANAL-20-290</td>
<td>Eonycteris</td>
<td>spelaea</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
<td>99%</td>
</tr>
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<td>Eonycteris</td>
<td>spelaea</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
<td>97%</td>
</tr>
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<td>Eonycteris</td>
<td>spelaea</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
<td>99%</td>
</tr>
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<td>Eonycteris</td>
<td>spelaea</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
<td>99%</td>
</tr>
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<td>Eonycteris</td>
<td>spelaea</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
<td>99%</td>
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<td>Cynopterus</td>
<td>sphinx</td>
<td>Betacoronavirus</td>
<td>Nobecovirus</td>
<td>Rousettus bat coronavirus GCCDC1</td>
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<td>Myotis</td>
<td>sp.</td>
<td>Alphacoronavirus</td>
<td>Pedacovirus</td>
<td>Bat alphacoronavirus*</td>
<td>99%</td>
</tr>
</tbody>
</table>

N=539 rectal swabs

Vincent Lacoste
IP-Laos (former member)
**Betacoronavirus enrichment**

- *In-silico probe design* from nucleotide sequence alignment of all *Betacoronavirus* subgenera
  - 13-mer primers pan-generic or specific to each subgenus (MSSPE method) 
    - Deng X. et al., 2020 Nat. Microbiol. doi: 10.1038/s41564-019-0637-9
  - 432 primers

1. Reverse transcription with **spiked + random** primers (10/1)
2. Second-strand synthesis
3. Library preparation with or without random pre-amplification
Evolution history of bat CoVs

- **At the whole genome level**
  - BANAL viruses close to SARS-CoV-2 early strains
  - High degree of conservation between CoVs carried by different *Rhinolophus* species living in sympatry

- **Similarity along the genome**
  - BANAL viruses present >95% nt identity along the genome, BANAL-52 the closest known to date
  - In the spike, BANAL viruses closer to SARS-CoV-2 than bat and pangolin CoVs
  - No furin site
Numerous recombinations in SARS-CoV-2 history

- No pangolin CoV as direct contributor
- One / multiple ancestors depending on the fragment of genome
- In the spike: RaTG13 CoV for the NTD, BANAL CoVs for RBD and N-terminal part of S2 (including the furin site)
Bat CoV RBD highly similar to SARS-CoV-2

- Alignment of the RBD region
  - BANAL-52 (R. malayanus) closer to SARS-CoV-2 than any bat / pangolin CoVs
Insights into RBD/hACE2 complex

- Determination of the crystal structure of BANAL RBDs

- Effects of mutations in RBD-hACE2 interactions
  - BANAL mutation $^H498Q$ disrupt transient hydrogen bonds but is known to increase affinity of SARS-CoV-2 RBD for hACE2 (Huang et al., 2021 EBioMedicine)
  - BANAL mutations $^K493Q$ → 2 additional salt bridges formed between BANAL-236 RBD & ACE2 → possible ↑ affinity

- Biolayer interferometry
  - $K_d$ BANAL viruses << SARS-CoV-2 → affinity of BANAL viruses compared to SARS-CoV-2 Wuhan strain
Cell entry through ACE2-dependent pathway

- **Lentiviral pseudoparticles expressing Wuhan or BANAL-236 wild type spikes**
  - Entry in 293T cells expressing hACE2
  - Entry blocked by SARS-CoV-2 neutralizing sera

- **BANAL-236 virus isolation**
  - Infection VeroE6 expressing endogenous ACE2
  - Infection blocked by soluble ACE2 (-3,6 log_{10}) similar to Wuhan (-3,8 log_{10})
Virus isolation

BANAL-236 virus growth
- Efficient replication in VeroE6, similar yield compared to Wuhan
- Lower replication in human cells expressing endogenous ACE2
  - No CPE observed compared to Wuhan
Ongoing studies

- **Pathogenicity in animal model (mice + primates)**
  - Level of replication in classical or humanized mice
  - Virus tropism in different organs
  - Adaptation mutations
  - Immune response to infection
  - Cross-protection against SARS-CoV-2
  - etc...

- **Infection of Laotian populations?**
  - In 2020, low seroprevalence in humans
    - Impact of governmental measures?
    - Young age population?
    - Cross-protective immunity?
  - Serosurvey of general vs bat-exposed populations
    - LIPS: RBD, N, full S
    - PNT

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Virachith et al., 2021 Lancet Reg Health West Pac.
Conclusions

- **SARS-CoV-2 origin**
  - Recombination from different *Rhinolophus* CoVs living in the limestone cave system of S-E. Asia
    - Rich diversity of bats living in sympatry ➔ exchanges of viruses
    - Major route of transmission: feces (but oral swabs also >0 for BANAL-27)
  - No need for an intermediate host to increase affinity for ACE2, but...
    - Origin of the furin cleavage site?
  - Route of introduction to the human population?

- **Risk of emergence of other bat CoVs?**
  - Exposure of guano collectors, religious communities, tourists?
  - If yes, symptomatic infection? Cross-protection against SARS-CoV-2?
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Institut Pasteur du Laos

Faculty of Environmental Sciences
National University of Laos
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