

# S.W.A.B. (Spontaneous Wildlife Autonomous Biosampler)

## An innovative tool for non-invasive contact-free wildlife health, pathogen, and welfare monitoring at landscape scales

**Authors:** Mariana M. Furtado<sup>1</sup>, Aspen C. F. Gonçalves<sup>1</sup>, Diana H.N. Blazquez<sup>1</sup>, Zubin K. Guha<sup>2</sup>, Washington C. Agostinho<sup>1</sup>, Vanessa Ezenwa<sup>2</sup>, Dawn Zimmerman<sup>2,3</sup>, Serap Aksoy<sup>2</sup>, Aaron Dollar<sup>2</sup>, Paulo Brandão<sup>1</sup>, Maristela Martins de Camargo<sup>1</sup>

**Affiliations:** <sup>1</sup>University of São Paulo (Brazil), <sup>2</sup>Yale University (USA), <sup>3</sup>VIEW Veterinary Initiative for Endangered Wildlife

### Background

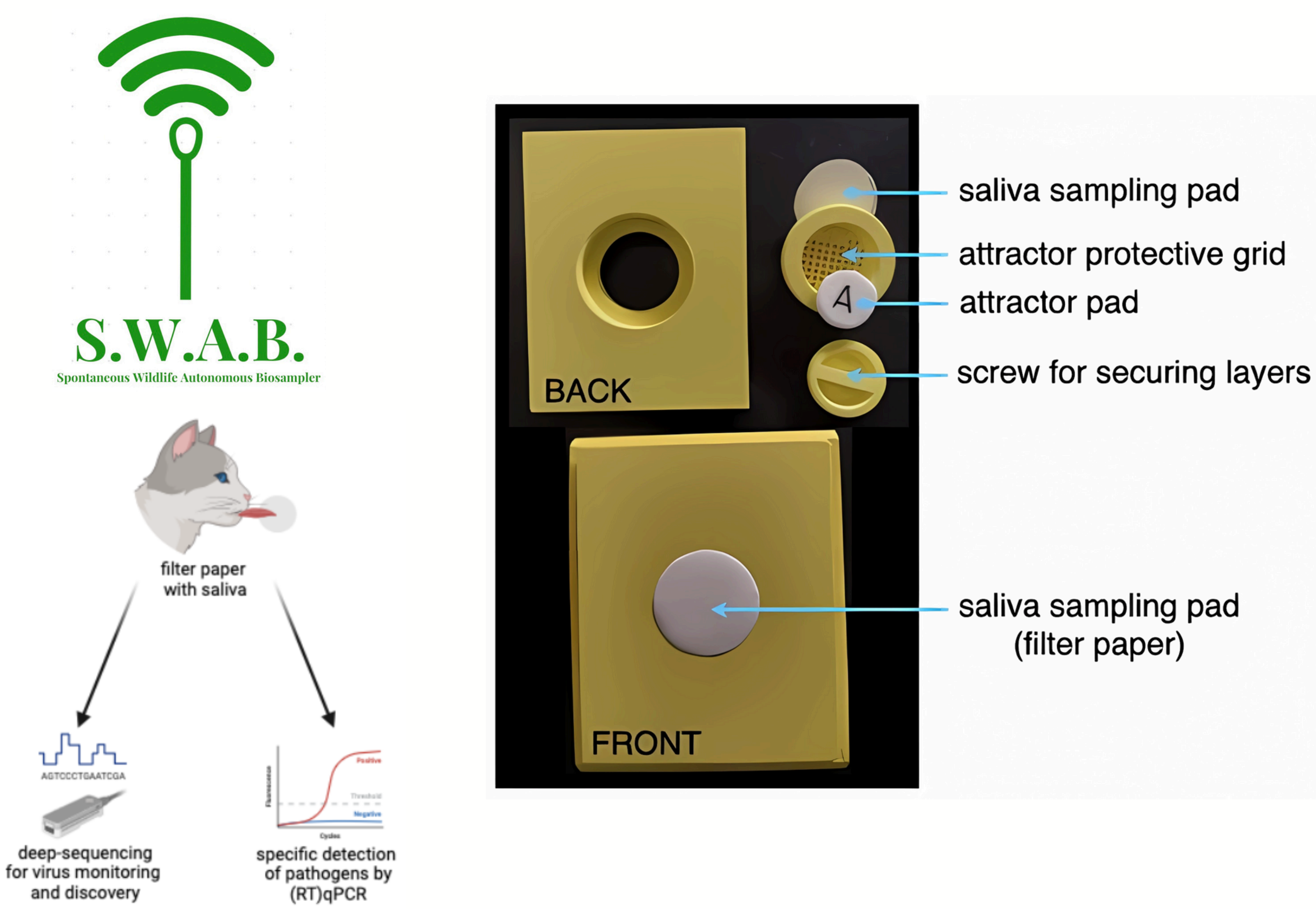
- The collection of biological samples has a high risk of morbidity and mortality for wild animals, as well as exposure risks for humans.
- Current non-invasive methods are limited to human-habituated animals, and/or are methods that require researchers to be on site to immediately collect the samples and identify the sampled animal.

### Objectives

- S.W.A.B. is an innovative and unique method to safely and autonomously acquire saliva samples from wildlife as sentinels for infectious diseases at high-risk remote locations.
- S.W.A.B. allows rapid and cost-effective pathogen risk assessment that can help design countermeasure strategies to protect wildlife, domesticated animals, and humans.

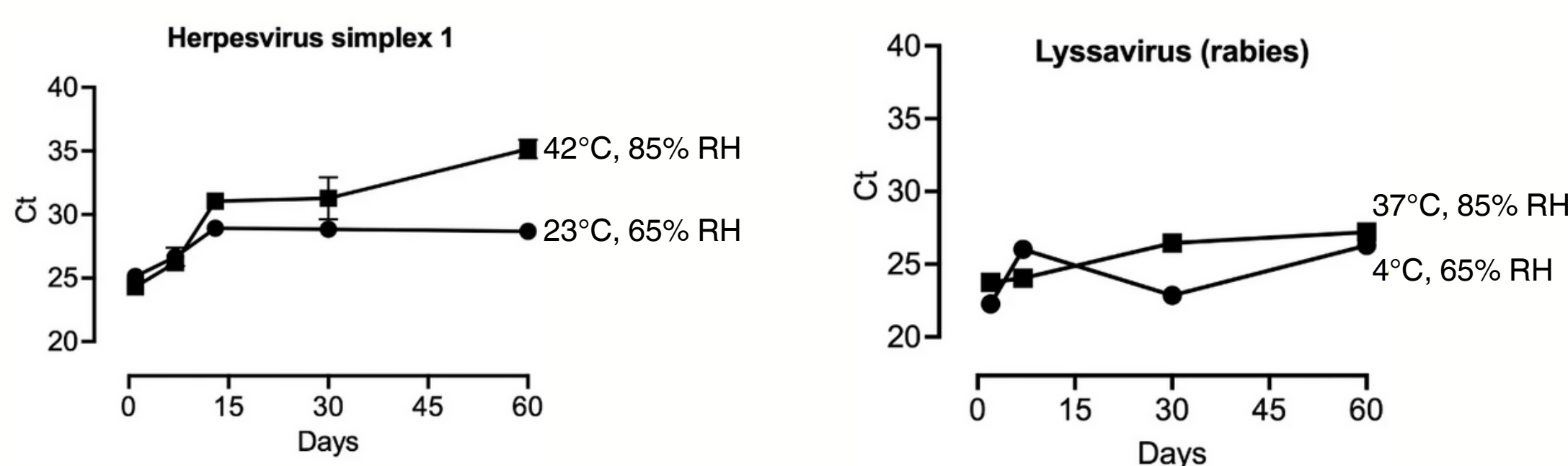
### Methods

- S.W.A.B. is a disposable 3D-printed collector containing an attractant odor and a sampling pad that is licked by the animal.
- S.W.A.B. prototypes were tested in forested areas around the University of São Paulo campus in Brazil, in domestic cats shelters, and in mice in a laboratory setting.
- Samples were tested for the presence of RNA viruses using (RT)qPCR and third generation (Oxford Nanopore Technology) sequencing.

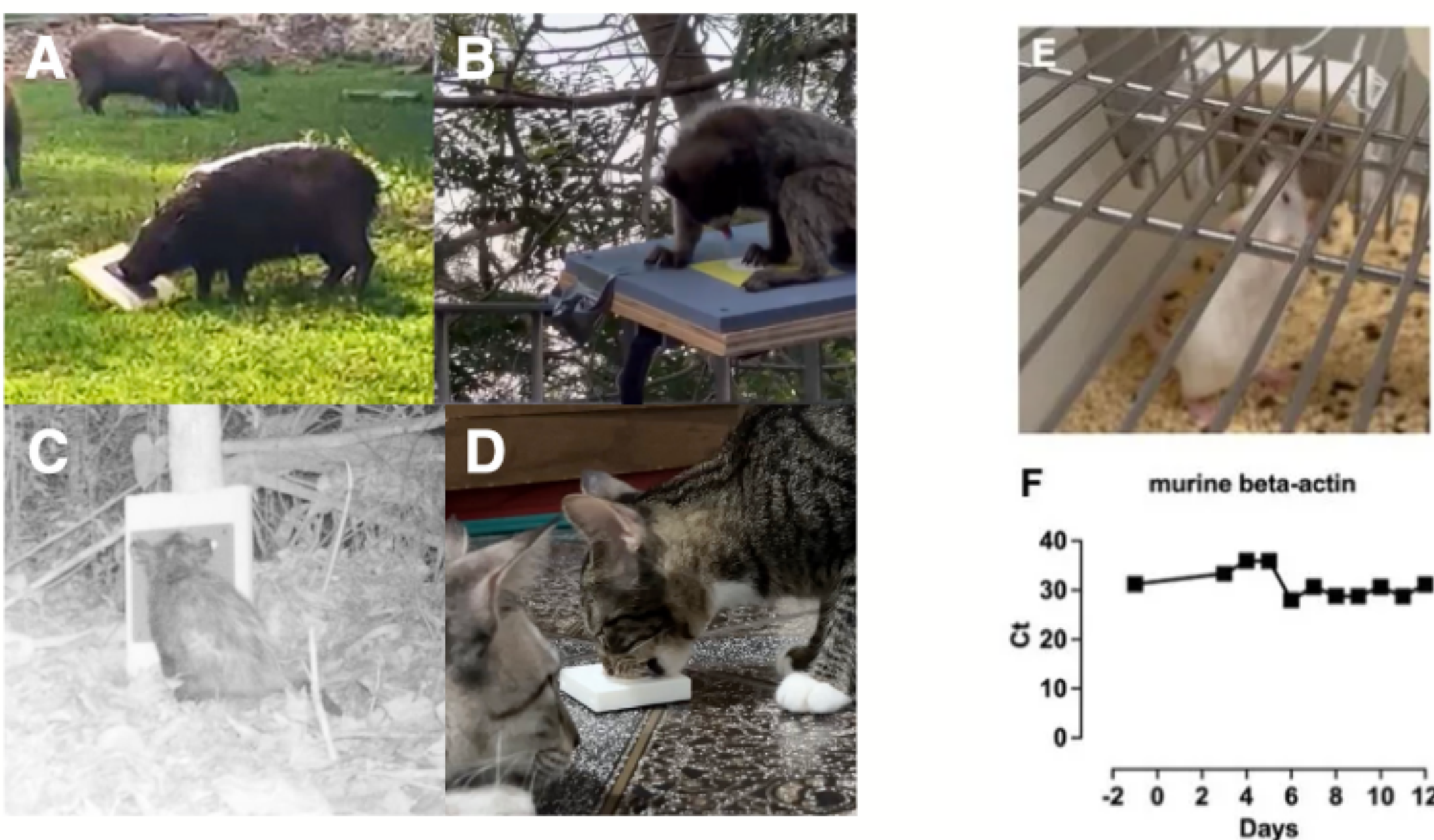


### Results

- RNA (rabies lyssavirus) and DNA (herpesvirus simplex 1/2) viruses could be detected after 60 days of being spiked on the sampling pad and kept at different storage conditions in the laboratory.



- Capybaras (A), marmosets (B), opossums (C), cats (D), horses (*not shown*), and laboratory mice (E) successfully interacted with S.W.A.B. prototypes. (F) Murine beta-actin mRNA was detected daily on licked sampling pads.

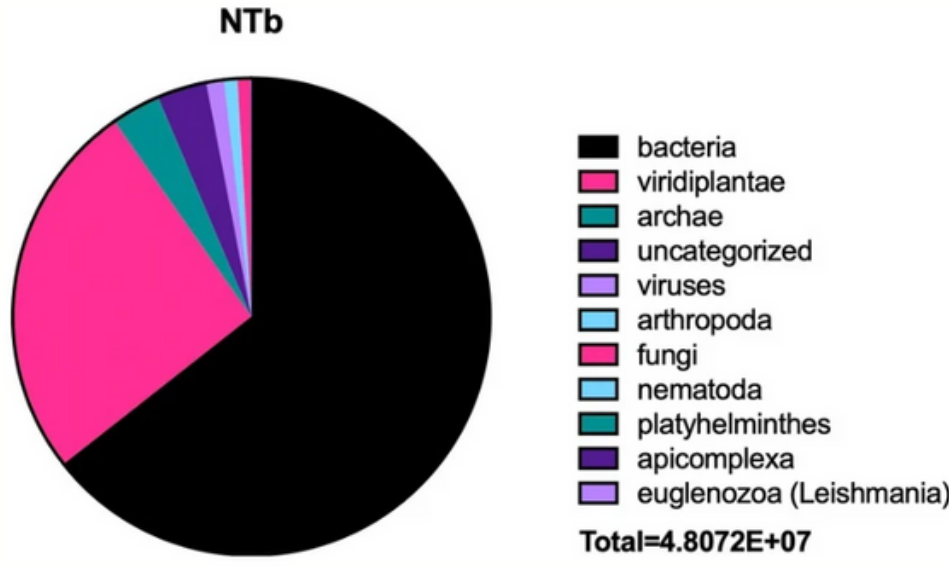


- Salivary samples deposited on sampling pads were more sensitive than oropharyngeal swabs (6 positive samples versus 4) and identified two cats as feline leukemia virus (FeLV)-positive that had tested serologically negative (SNAP test, Idexx) (*not shown*).
- Nanopore metagenomics of RNA from licked paper samples detected one cat actively shedding FeLV particles.

**Table 1 -** Detection of FeLV RNA (shedding viruses) and DNA (proviral DNA) in saliva from 10 shelter cats by (RT)qPCR or Nanopore™ metagenomics. nd = not detected. \* weak interaction with paper.

| Cat       | (RT)qPCR oral swab<br>RNA + DNA | (RT)qPCR licked paper<br>RNA + DNA | Nanopore™ metagenomics licked paper<br>RNA only |
|-----------|---------------------------------|------------------------------------|-------------------------------------------------|
| A         | nd                              | 34.3                               | nd                                              |
| B         | nd                              | nd                                 | nd                                              |
| C         | 23.6                            | nd*                                | nd                                              |
| D         | 20.17                           | 25.7                               | POSITIVE                                        |
| M         | 18.64                           | 34.1                               | nd                                              |
| P1        | nd                              | 34.4                               | nd                                              |
| P2        | 22.06                           | 30.6                               | nd                                              |
| S         | nd                              | 35.0                               | nd                                              |
| T         | nd                              | nd                                 | nd                                              |
| W         | nd                              | nd                                 | nd                                              |
| FeLV ctrl | 21.0                            | 21.4                               | POSITIVE                                        |

- Nanopore metagenomics of RNA proved useful for discovery of environmental contaminants, including diet components, insects, and circulating pathogens.



| Table 2 - Examples of pathogens found by sequencing of paper pads licked by shelter cats |                                   |                                |
|------------------------------------------------------------------------------------------|-----------------------------------|--------------------------------|
| <i>Aspergillus fumigatus</i>                                                             | <i>Cryptosporidium parvum</i>     | <i>Pasteurella multocida</i>   |
| <i>Aspergillus niger</i>                                                                 | <i>Haemophilus parainfluenzae</i> | <i>Sarcoptes scabiei</i>       |
| <i>Clostridioides difficile</i>                                                          | <i>Klebsiella pneumoniae</i>      | <i>Trichomonas vaginalis</i>   |
| <i>Clostridium botulinum</i>                                                             | <i>Neisseria gonorrhoeae</i>      | <i>Yersinia enterocolitica</i> |



### Conclusions

- Future steps include improving protocols for viral monitoring and discovery, validating paper samples for stress and welfare hormone measurements, evaluating safety of deployment in areas with large carnivores, and advancing the instrumentation for autonomous use.
- S.W.A.B. will allow assessment of pathogen spillover risk in remote areas, without the need of local trained personnel, sophisticated laboratories, or advanced wildlife handling capabilities.

- S.W.A.B. will facilitate interdisciplinary research within the same landscape, including monitoring vector-borne diseases that shift due to climate change and changing vector ranges, evaluating stress, welfare and reproductive hormones in endangered wildlife pressured by anthropogenic habitat degradation, characterizing genomic changes as environmental shifts alter sympatric biodiversity, and measuring changes in animals' immunity as a result of chronic stress.
- An open-sourced blueprint for S.W.A.B. will be made available, facilitating large-scale dissemination of the technology.
- Contact us if you would like to try S.W.A.B.

### Funding and Support



revive & restore  
GENETIC RESCUE OF ENDANGERED  
AND EXTINCT SPECIES



Why swab only a few individuals when you can S.W.A.B. a landscape?  
@swab\_biosampler  
@MaristelaMDC  
#GOHC #WOHC2024



linktr.ee/SWABbiosampler  
mmcamar@usp.br  
swab@usp.br