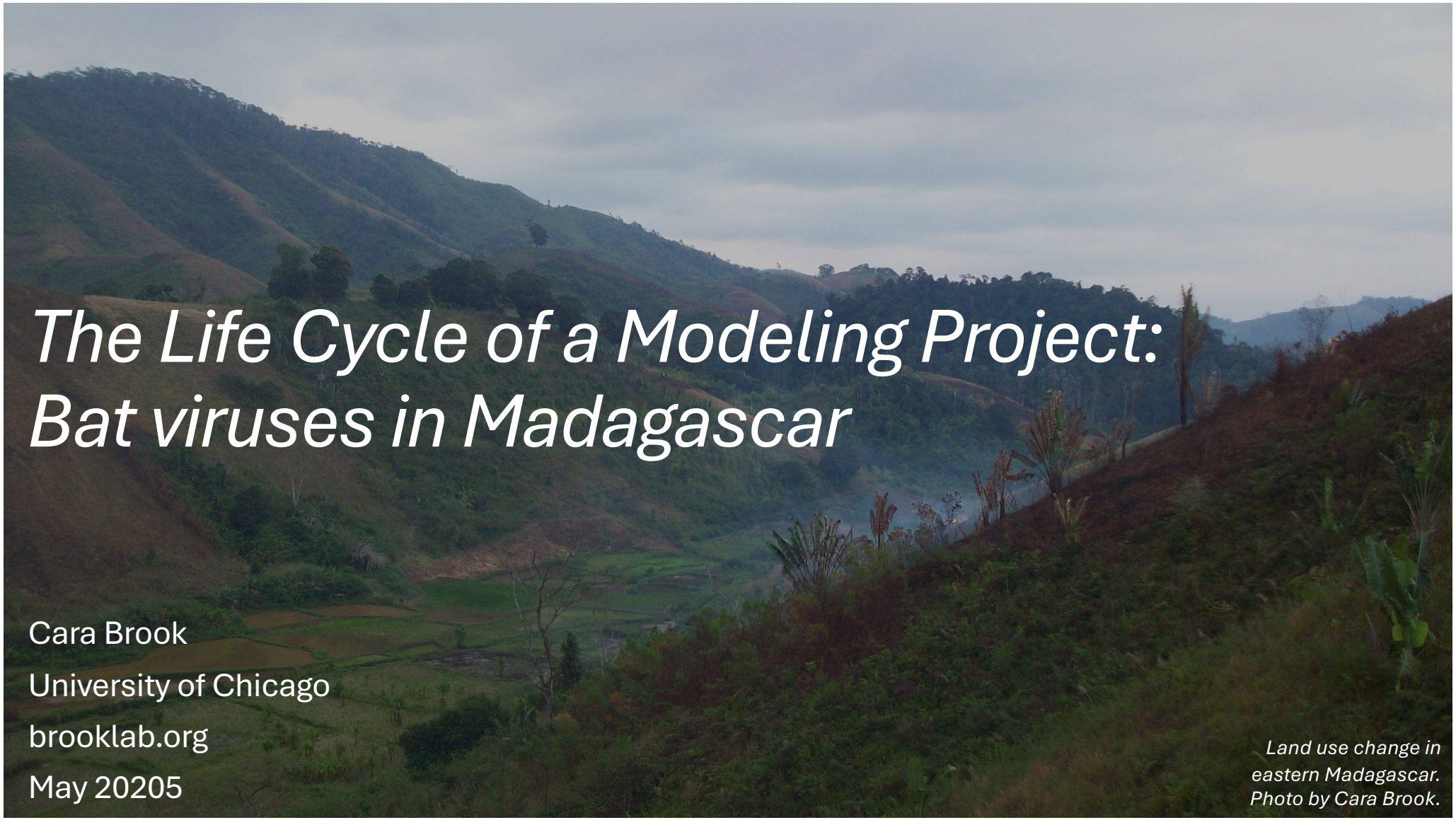


The Life Cycle of a Modeling Project: Bat viruses in Madagascar

Cara Brook
University of Chicago
brooklab.org
May 20205

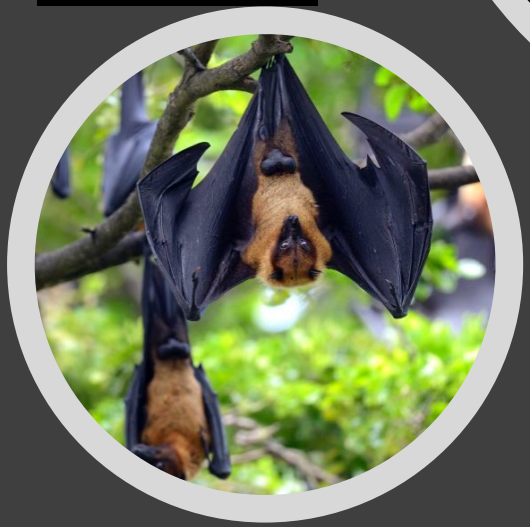
Land use change in eastern Madagascar. Photo by Cara Brook.

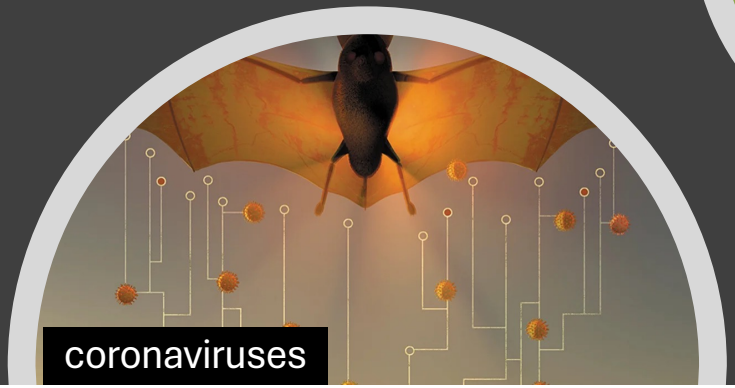
Bats are natural **reservoir hosts** for **zoonotic viruses** that cause **higher case fatality rates** in humans than do zoonoses derived from any other mammal or bird host.

henipaviruses

Guth et al. 2019. *Proc Roy Soc. B.*
Guth et al. 2022. *PNAS.*

lyssaviruses

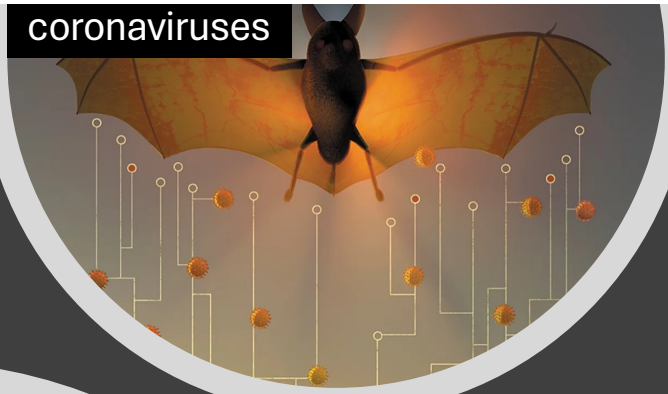


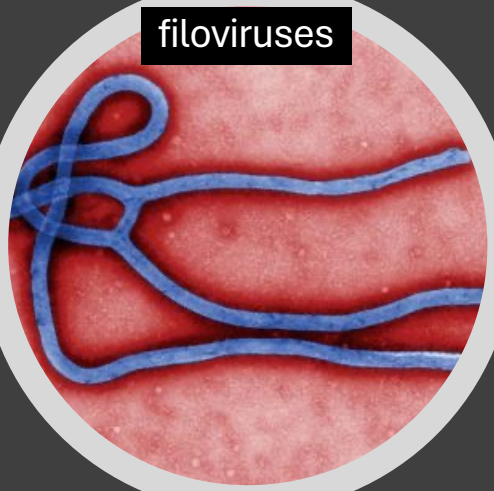


coronaviruses

filoviruses

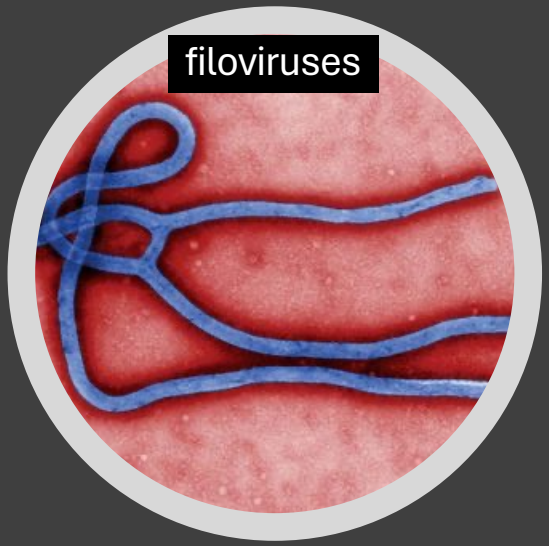
Sarah Guth,
Assistant Professor,
Skyline College

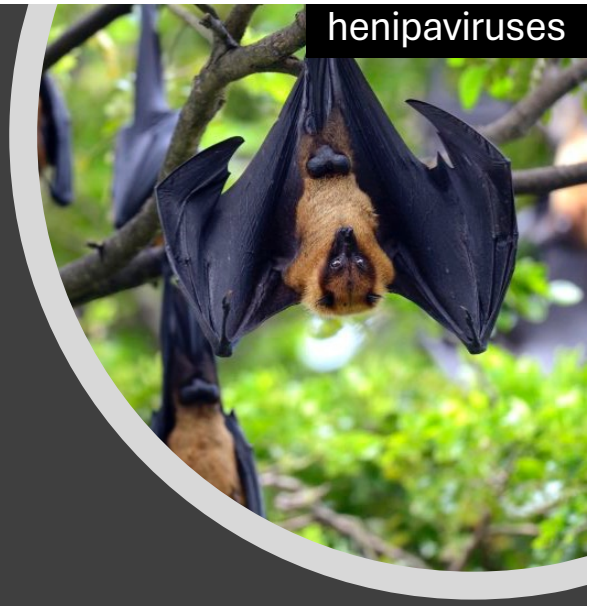




Seasonal cycles are observed for multiple bat virus zoonoses.

Brook & Dobson. 2015. *Trends Micro.*
Schountz et al. 2017. *Front Immunol*





Seasonal cycles are observed for multiple bat virus zoonoses.

The **majority of bat virus zoonoses** occur in the **winter**.

Brook & Dobson. 2015. *Trends Micro.*
Schountz et al. 2017. *Front Immunol*

Step 1: Define your research question ~

*What **factors** are **associated** with **zoonotic virus** infection in Madagascar fruit bats?*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 1: Define your research question ~

*What **factors** are **associated** with **zoonotic virus** infection in Madagascar fruit bats?*

statistical

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 1: Define your research question ~

*What **factors** are **associated** with **zoonotic virus** infection in Madagascar fruit bats?*

Step 2: Formulate a hypothesis ~

***Reproductive season** and **poor nutritional condition** are associated with **zoonotic virus** infection in Madagascar fruit bats.*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 3:
Collect data ~

We run a **longitudinal field project** tracking zoonotic virus dynamics in Malagasy fruit bats.

Photos by Cara Brook and Nikhil Deshmukh.

Christian Ranaivoson, UChicago Postdoc

Angelo Andrianiaina, UTana PhD Student

Martin Roland, Postbacc Field Tech

Santino Andry, UTana PhD Student

Monique Ades, Postbacc Field Tech

Rova Ratsimamanga, UTana MSc Student

Why **Madagascar**?

Why **Madagascar**?

Madagascar's **three endemic fruit bat species** are linked to both African and Asian viral zoonoses.

Eidolon dupreanum

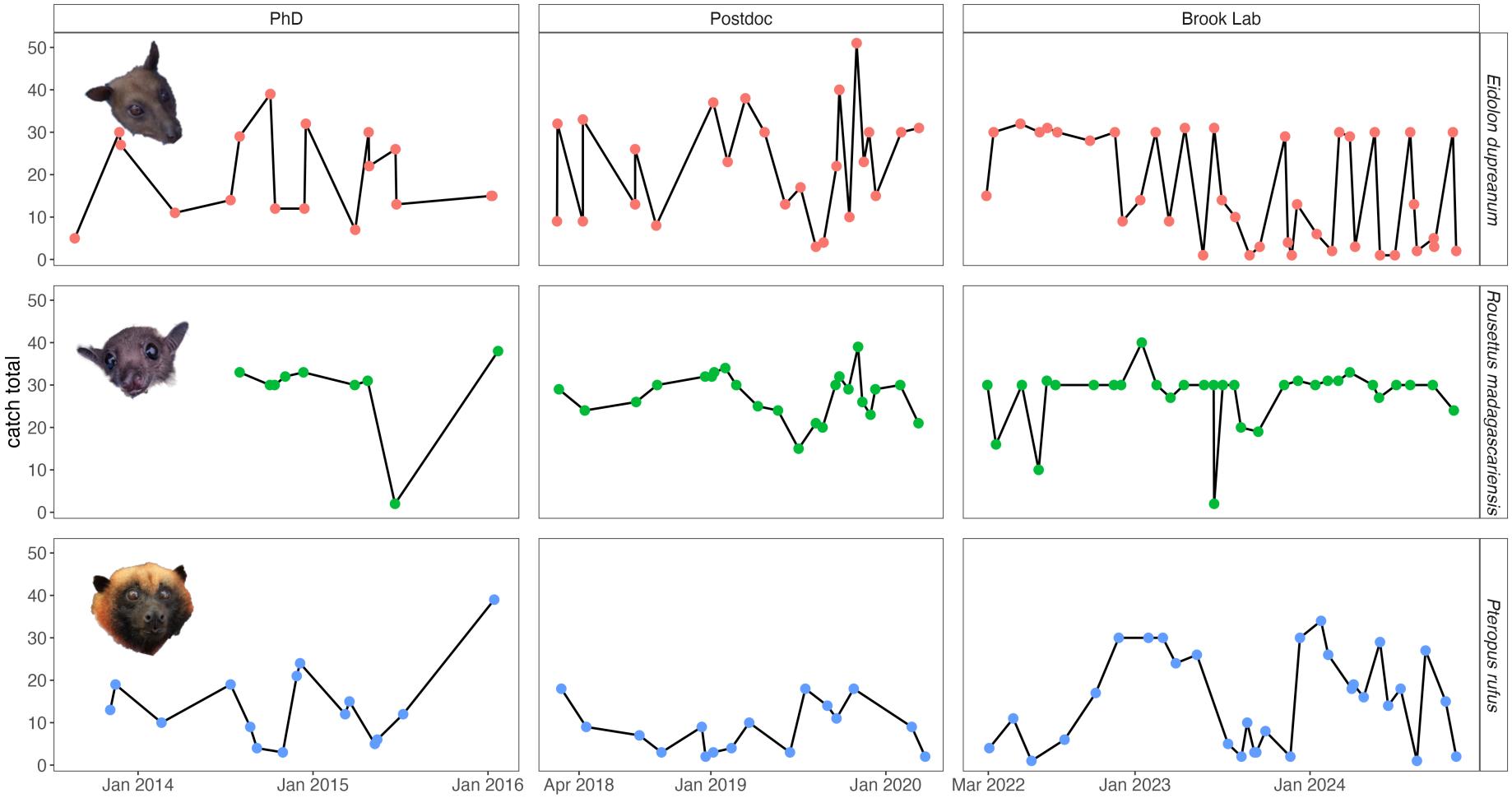
Rousettus madagascariensis

Pteropus rufus

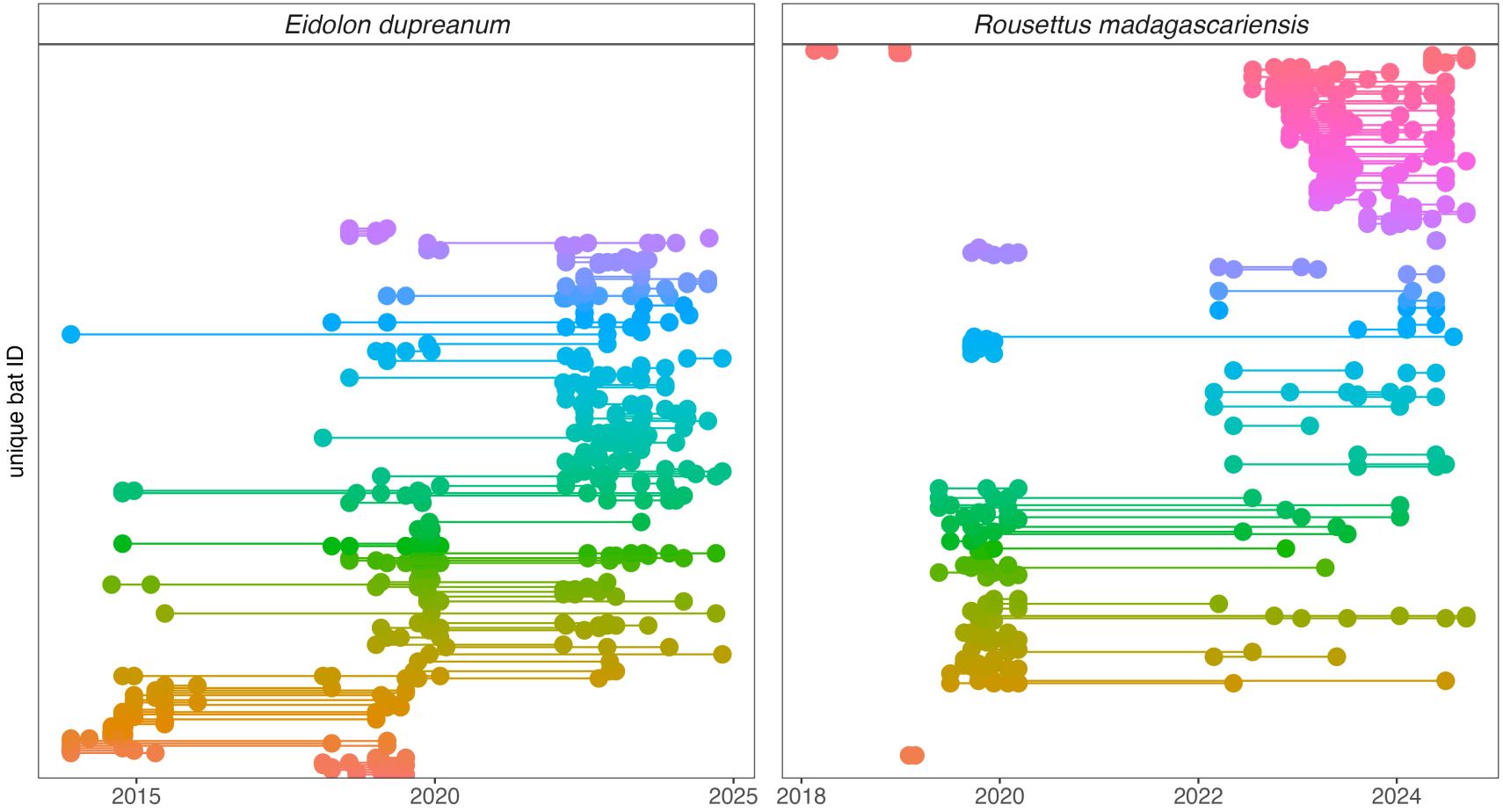
Madagascar fruit bats are **consumed** as a source of human food, posing risks for **zoonotic exposure** – and highlighting the **interplay of anthropogenic stressors** and **infection dynamics**.

Malagasy Flying Fox, *Pteropus rufus,* on the menu.
Photos by Cara Brook.

We **capture** and **collect samples** from fruit bats at **monthly** timepoints across Madagascar.



We apply **PIT tags** to all captures to enable monitoring of individuals through time.



PIT tag application in *R. madagascariensis*. Photo by Cara Brook.

In the laboratory, we **process field-collected samples** to build a time series of **molecular** and **serological** viral dynamics.

Ekipa Fanihy postbacc technicians Anecia Gentles & Kim Rivera with postdoc Dr. Christian Ranaivoson. Institut Pasteur of Madagascar. Photo by Cara Brook.

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Ekipa Fanihy postbacc technicians Anecia Gentles & Kim Rivera with postdoc Dr. Christian Ranaivoson. Institut Pasteur of Madagascar. Photo by Cara Brook.

feces →

urine →

saliva →

RNA is extracted, then undergoes PCR and sequencing to identify **active virus infection**

I

Infectious

In the laboratory, we **process field-collected samples** to build a time series of **molecular** and **serological** viral dynamics.

Ekipa Fanihy postbacc technicians Anecia Gentles & Kim Rivera with postdoc Dr. Christian Ranaivoson. Institut Pasteur of Madagascar. Photo by Cara Brook.

feces →

urine →

saliva →

RNA is extracted, then undergoes PCR and sequencing to identify **active virus infection**

I
Infectious

blood →

centrifuged to serum to identify **antibodies** signifying **past history of virus exposure**

R
Recovered

Using PCR and mNGS, we have discovered and described **numerous novel Malagasy bat viruses**, which highlight a **unique host-microbe coevolutionary history**.

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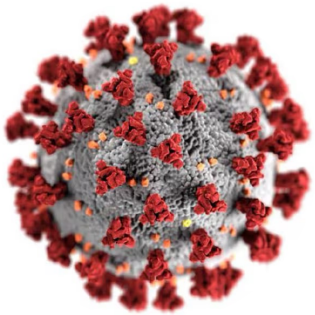
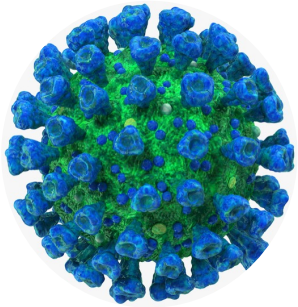
3 full-genome coronaviruses



Kettenburg et al. 2022. *Frontiers in Public Health*.

Gwen Kettenburg, PhD student

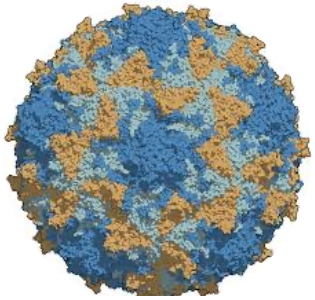
1 full-genome astrovirus



Horigan et al. 2024. *Virology Journal*.

Sophia Horigan, PhD student

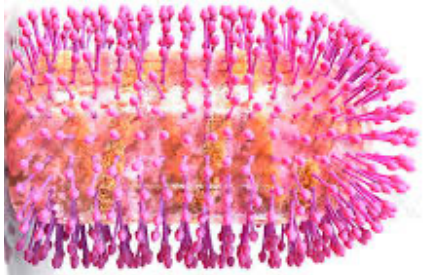
14 full-genome + 37 partial-genome picornaviruses



Kettenburg et al.
In final prep for *mBio*.
Gonzalez et al.
In Revision. *PLoS One*.

Freddy Gonzalez, NIH PREP scholar

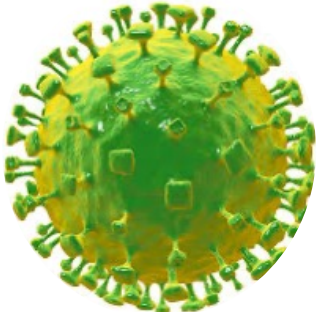
1 partial-genome lyssavirus



Kettenburg et al. In final prep for *Emerging Infectious Diseases*.

Gwen Kettenburg, PhD student

2 full-genome + 2 partial-genome henipaviruses

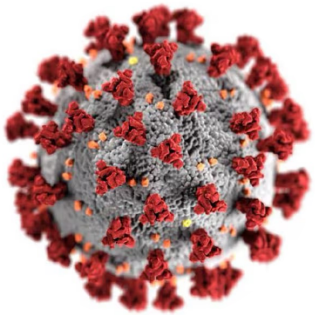


Madera et al. 2022 *J of Virology*.
Kettenburg et al. In prep.

Sharlene Madera, UCSF Postdoc

Using PCR and mNGS, we have discovered and described **numerous novel Malagasy bat viruses**, which highlight a **unique host-microbe coevolutionary history**.

3 full-genome coronaviruses



Kettenburg et al. 2022. *Frontiers in Public Health*.

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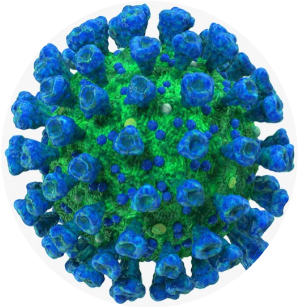

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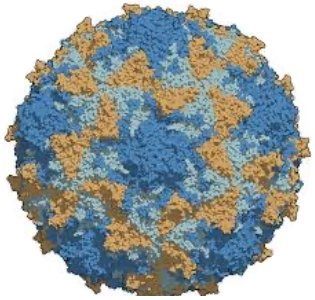

Kettenburg et al. In final prep for *mBio*. Gonzalez et al. In Revision. *PLoS One*.

Freddy Gonzalez, NIH PREP scholar

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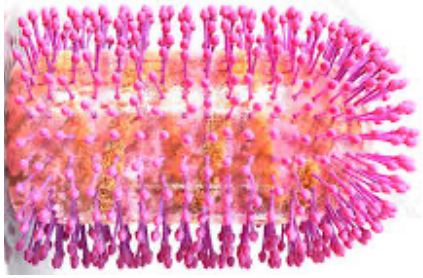

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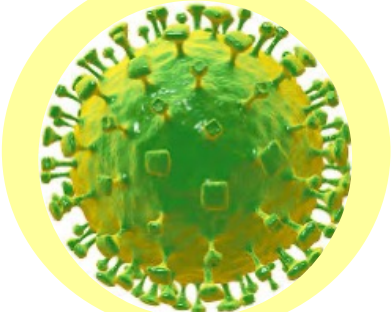
Gwen Kettenburg, PhD student

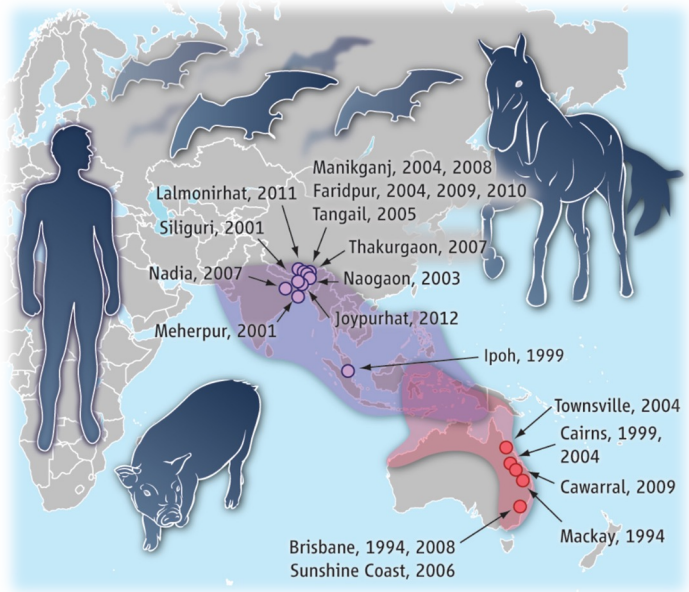
2 full-genome + 2 partial-genome henipaviruses

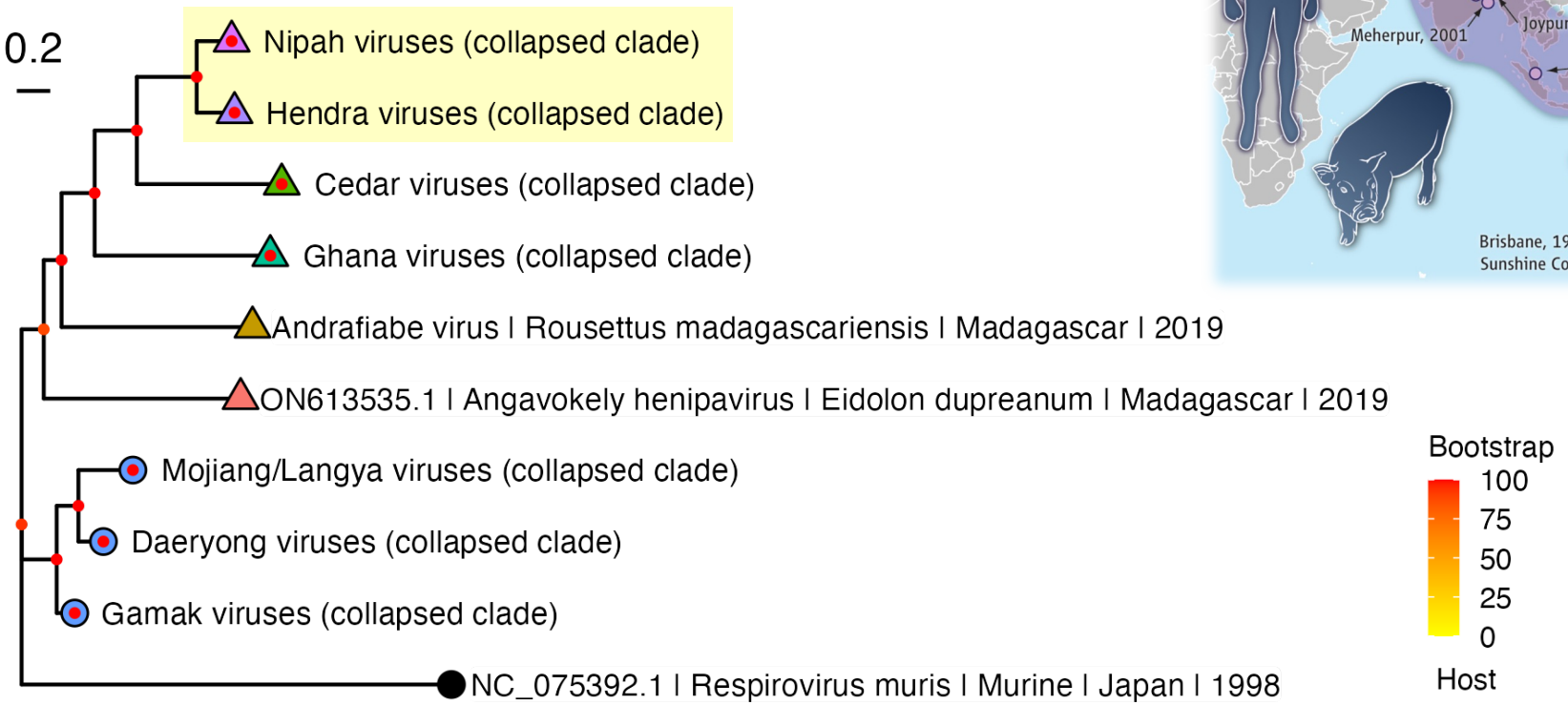


Madera et al. 2022 *J of Virology*. Kettenburg et al. In prep.

Sharlene Madera, UCSF Postdoc

Henipaviruses (HNVs) are best known by the canonical, zoonotic Hendra and Nipah virus, both with bat origins.

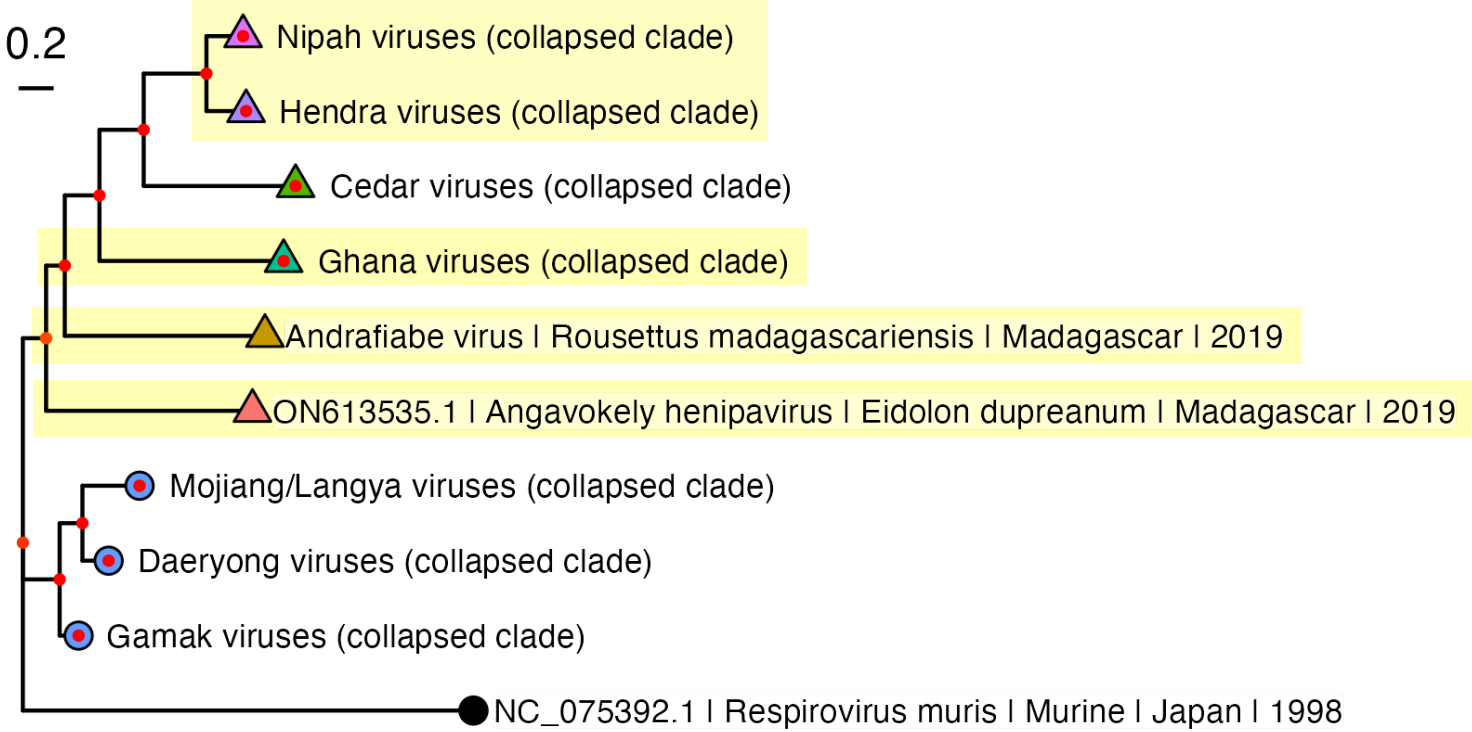




Maximum Likelihood Full-Genome Nucleotide Phylogeny (GTR+I+G4)

Messling and Cattaneo 2012. *Science*.

Field work and sequencing have identified **four clades of HNV** circulating in **wild Madagascar fruit bats**.



Bootstrap
100
75
50
25
0

Host
Bat host
Non-bat host

Maximum Likelihood Full-Genome Nucleotide Phylogeny (GTR+I+G4)

Kettenburg et al. In prep.
Madera et al. 2022 *J of Virology.*

Christian Ranaivoson,
UChicago Postdoc

Gwen Kettenburg,
UChicago PhD student

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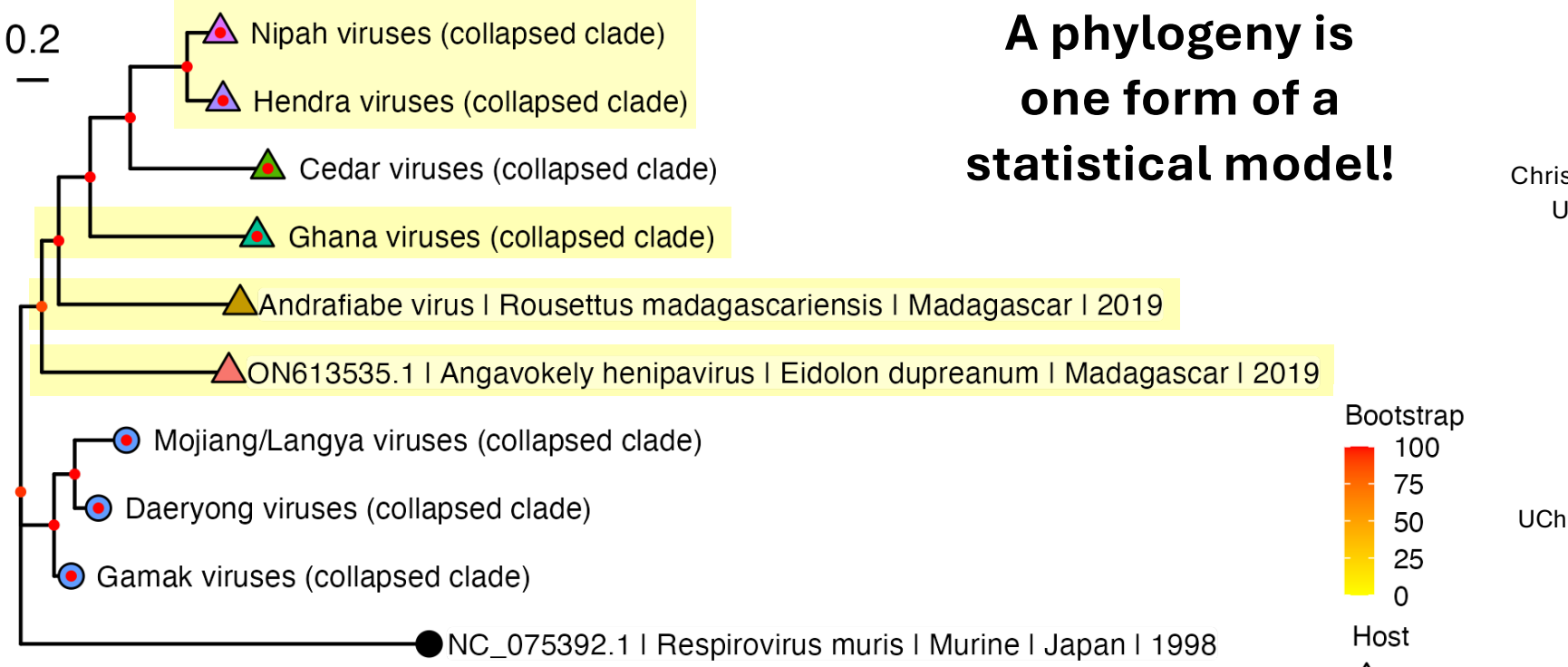

A phylogeny is one form of a statistical model!

Christian Ranaivoson, UChicago Postdoc

Gwen Kettenburg, UChicago PhD student

Kettenburg et al. In prep.
Madera et al. 2022 *J of Virology.*

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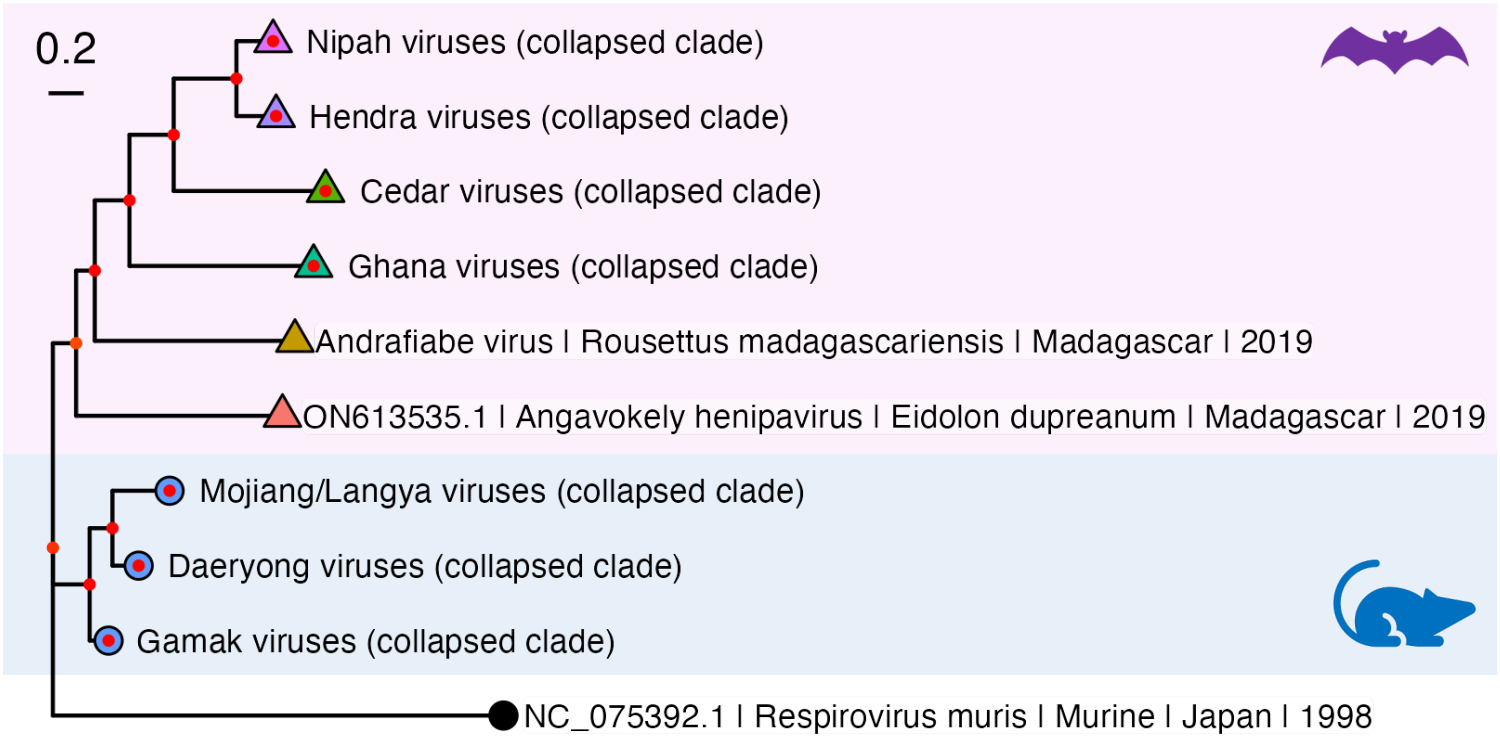

Kettenburg et al. In prep.
Madera et al. 2022 *J of Virology.*

Maximum Likelihood Full-Genome Nucleotide Phylogeny (GTR+I+G4)

Malagasy viruses represent the **most basal bat HNVs** described to date.

Christian Ranaivoson, UChicago Postdoc

Bootstrap
100
75
50
25
0

Host
Bat host
Non-bat host

Gwen Kettenburg, UChicago PhD student

Step 1: Define your research question ~

*What **factors** are **associated** with **henipavirus** infection in Madagascar fruit bats?*

Step 2: Formulate a hypothesis ~

***Reproductive season** and **poor nutritional condition** are associated with **henipavirus** infection in Madagascar fruit bats.*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 4: Construct a model that demonstrates your hypothesis ~

Hyp 1: ***Season*** *and* ***nutritional condition*** *are associated with* ***henipavirus*** *infection in Madagascar fruit bats.*

Hyp 1:

```
gam(seropos_YN~ s(mass_forearm_residual) +
      s(day_of_year) + s(year, bs="re"),
      family = "binomial", data=Eid.dat)
```

Hyp 2: ***Sex-specific season*** *and* ***sex-specific nutritional condition*** *are associated with* ***henipavirus*** *infection in Madagascar fruit bats.*

Hyp 2:

```
gam(seropos_YN~
      s(mass_forearm_residual, by ="sex") +
      s(day_of_year, by = "sex") + s(year, bs="re"),
      family = "binomial", data=Eid.dat)
```

Step 5: Assess model fit to the data ~

Hyp 1: ***Season*** *and* ***nutritional condition*** *are associated with* ***henipavirus*** *infection in Madagascar fruit bats.*

```
> summary(m1GhVnoSeX)

Family: binomial
Link function: logit

Formula:
seropos ~ s(doy_corrected, k = 7, bs = "cc") + s(mass_forearm_residual,
    k = 7, fx = FALSE, bs = "cr") + s(year, bs = "re")

Parametric coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  -0.6740     0.1796  -3.752 0.000175 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Approximate significance of smooth terms:
                           edf Ref.df Chi.sq p-value
s(doy_corrected)         1.788      5  8.571  0.0124 *
s(mass_forearm_residual) 3.246      6  8.134  0.0431 *
s(year)                  1.060      2  2.378  0.0969 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

R-sq.(adj) =  0.0304   Deviance explained = 3.35%
UBRE = 0.29551  Scale est. = 1         n = 503
```

Step 5: Assess model fit to the data ~

Hyp 2: ***Sex-specific season*** *and* ***sex-specific nutritional condition*** *are associated with* ***henipavirus*** *infection in Madagascar fruit bats.*

```
> summary(m1GhVnoSeX)

Family: binomial
Link function: logit

Formula:
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```

Step 5: Assess model fit to the data ~

Hyp 1:

gam(seropos_YN~
s(mass_forearm_residual) + s(day_of_year),
family = “binomial”, data=Eid.dat)

```
> AIC(m1GhVnoSeX, m1GhVSeX)
                 df      AIC
m1GhVnoSeX 7.094527 651.6397
m1GhVSeX   8.269294 650.5762
```

Hyp 2:

gam(seropos_YN~
s(mass_forearm_residual, by =“sex”) +
s(day_of_year, by = “sex”),
family = “binomial”, data=Eid.dat)

Step 5: Assess model fit to the data ~

Hyp 1:

```
gam(seropos_YN~
      s(mass_forearm_residual) + s(day_of_year),
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```

```
> AIC(m1GhVnoSeX, m1GhVSeX)
                 df      AIC
m1GhVnoSeX 7.094527 651.6397
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```

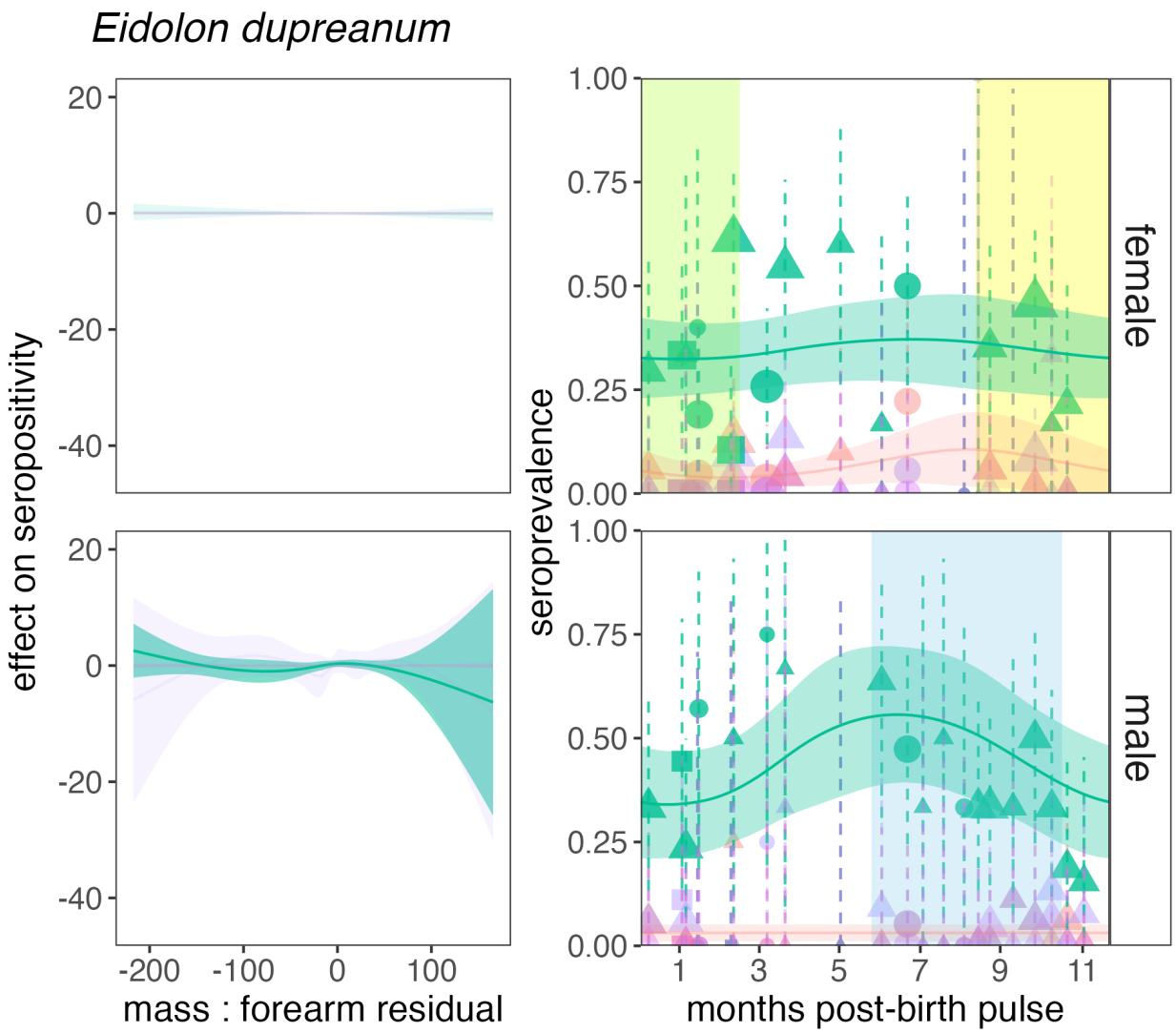
Hyp 2:

```
gam(seropos_YN~
      s(mass_forearm_residual, by ="sex") +
      s(day_of_year, by = "sex"),
      family = "binomial", data=Eid.dat)
```

Step 6: Optimize model parameters to best fit the data ~

- *Sex-specific seasonality is significantly associated with seropositive status in* E. dupreanum *fruit bats.*

- *Male nutritional condition (mass: forearm residual) is negatively correlated with seropositive status.*



Step 1: Define your research question ~

What drives seasonal ***henipavirus*** *cycles in* ***wild fruit bats*** *in Madagascar?*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 1: Define your research question ~

*What **drives** seasonal **henipavirus** cycles in **wild fruit bats** in Madagascar?*

mechanistic

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 1: Define your research question ~

*What **drives** seasonal **henipavirus** cycles in **wild fruit bats** in Madagascar?*

Step 2: Formulate a hypothesis ~

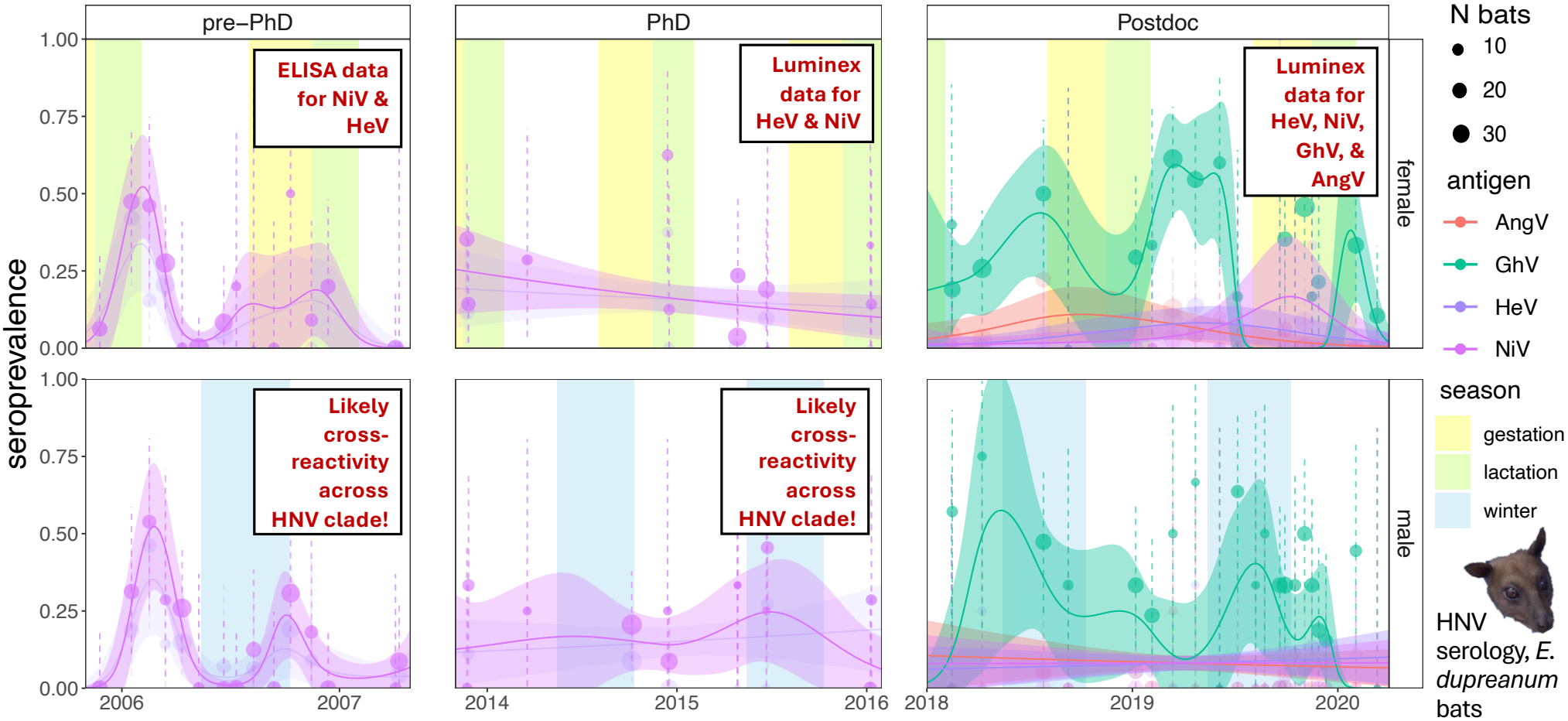
***Seasonal birth pulses** drive **zoonotic virus cycles** in Madagascar fruit bats.*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 3:

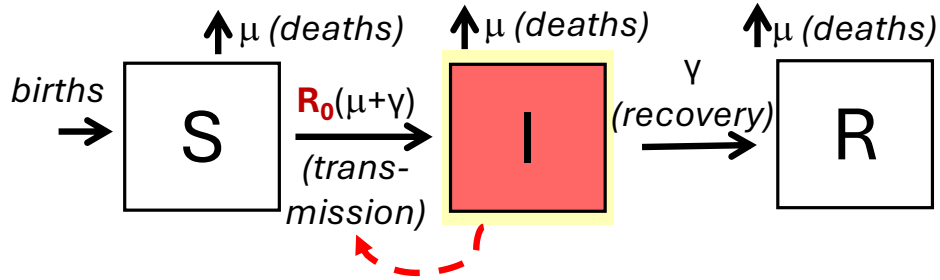
Collect data ~

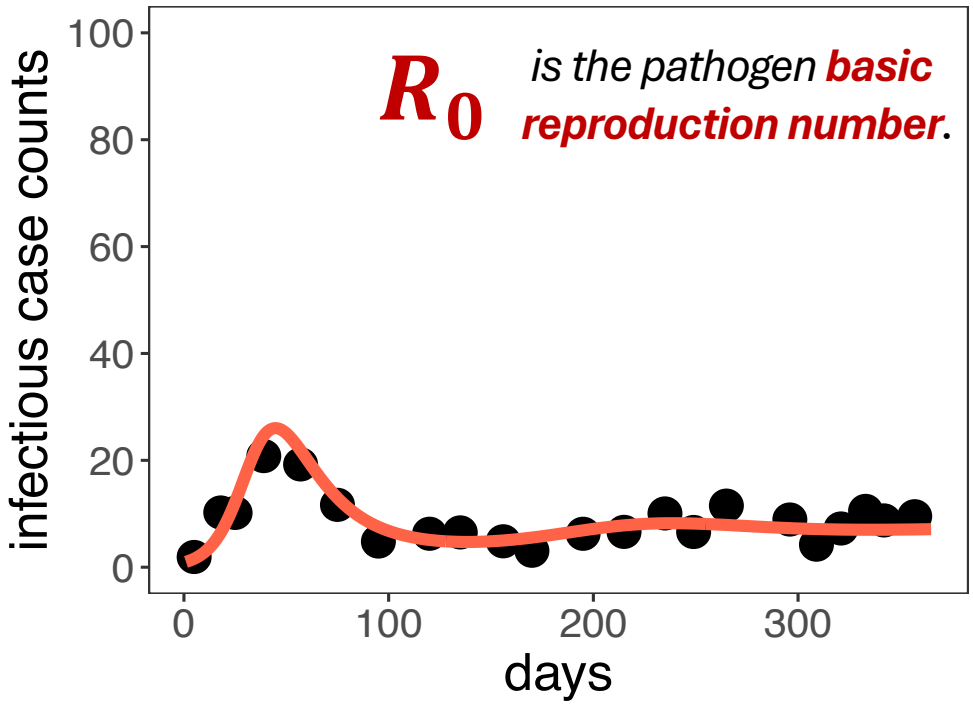
Field data show clear evidence of **seasonal cycles** of **henipaviruses** in Malagasy fruit bats.



HNV serology, *E. dupreanum* bats

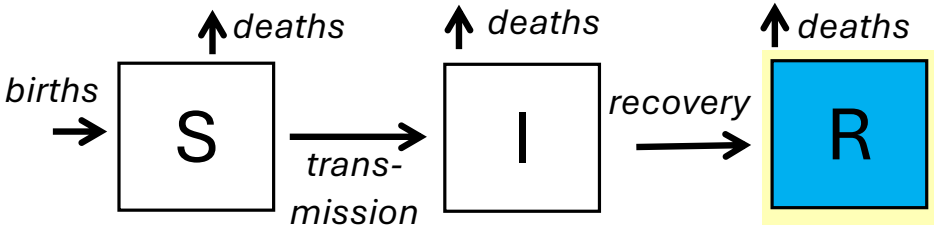
In public health, we can **estimate epidemic trajectories** from infectious case count data.

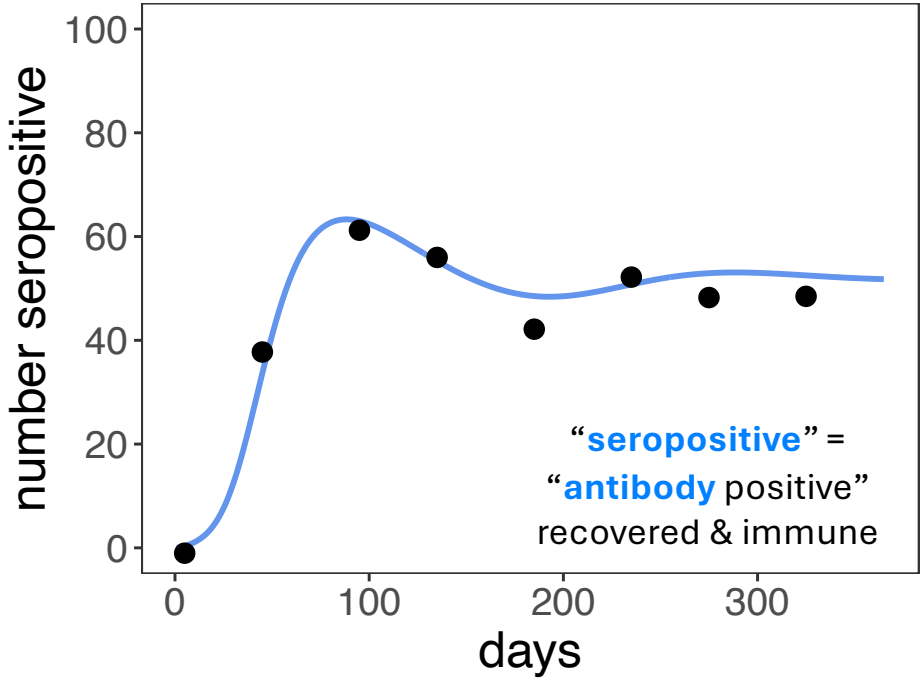




(epidemics grow @R_0>1 and decline @R_0<1)

In wildlife systems, data are often limited to **cross-sectional**, **serological** sampling.

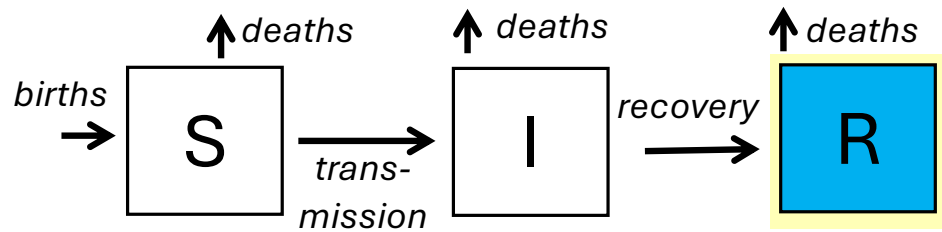


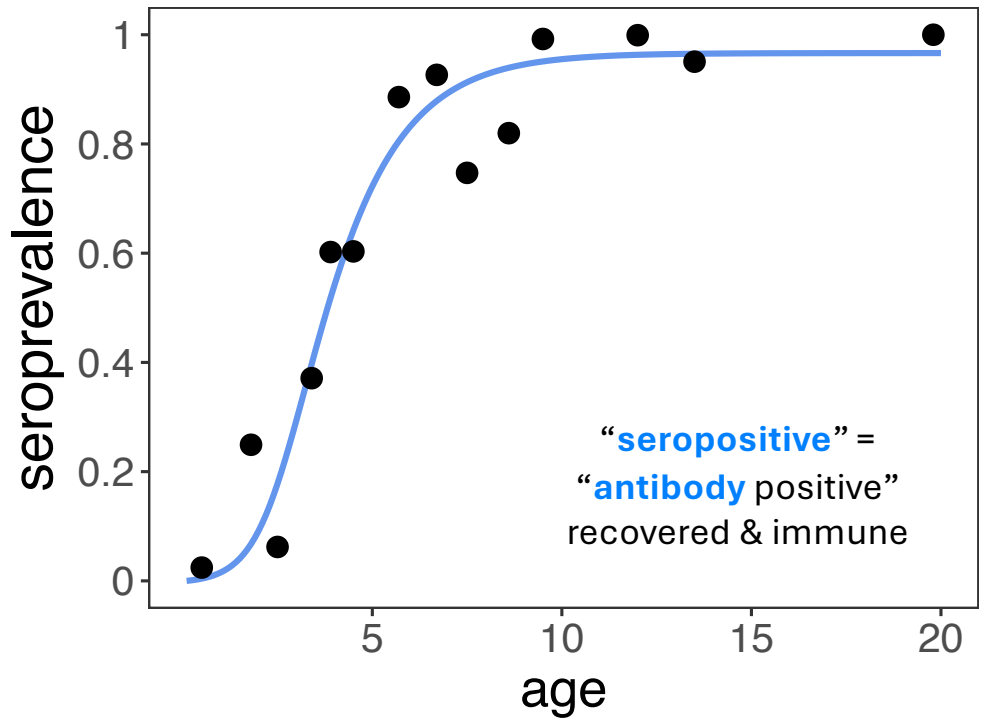


Kermack and McKendrick 1927 *Proc Roy Soc A*

b = birth rate
μ = death rate
β = transmission rate
γ = recovery rate

For wildlife systems, incorporation of **age structure** improves our inference from **seroprevalence** data.





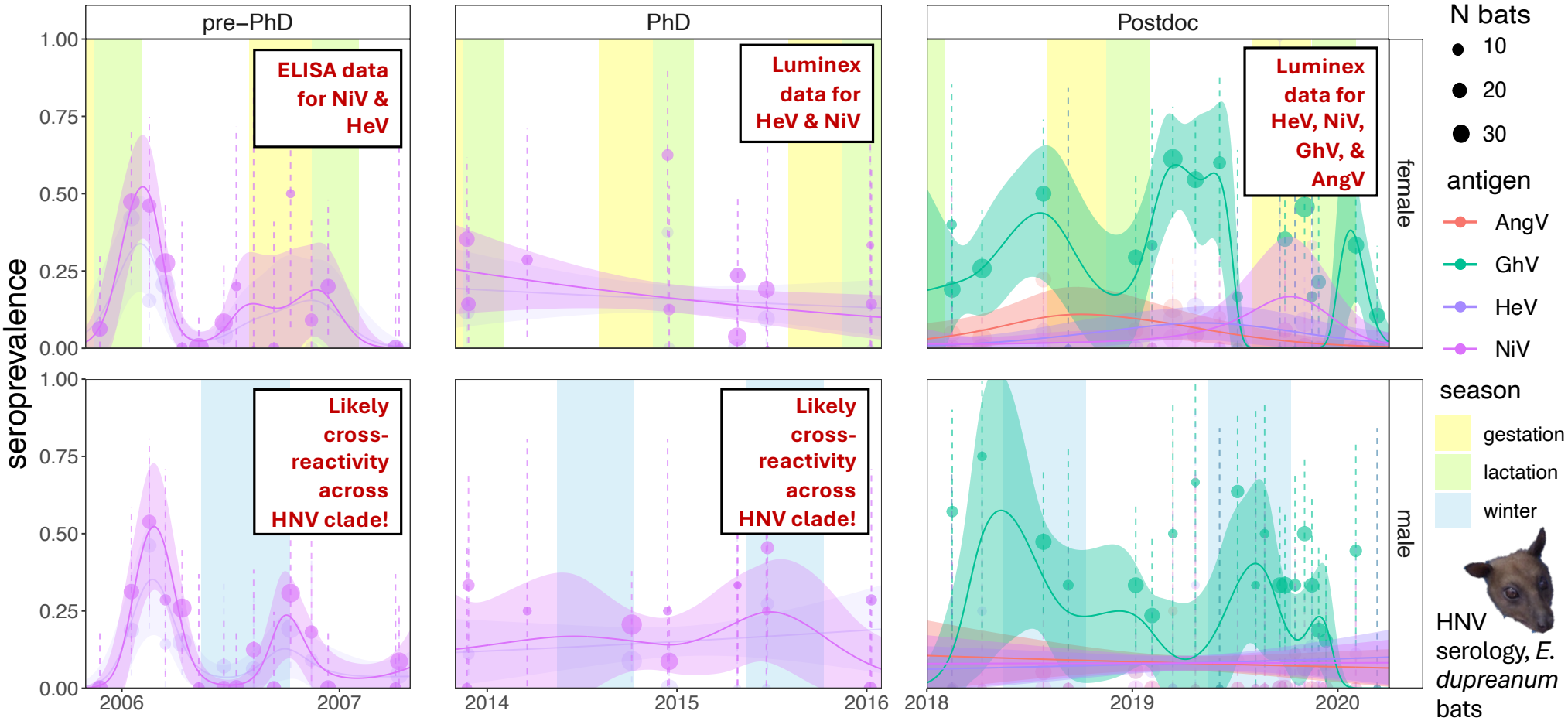
Kermack and McKendrick 1927 *Proc Roy Soc A*

b = birth rate
μ = death rate
β = transmission rate
γ = recovery rate

Step 3:

Collect data ~

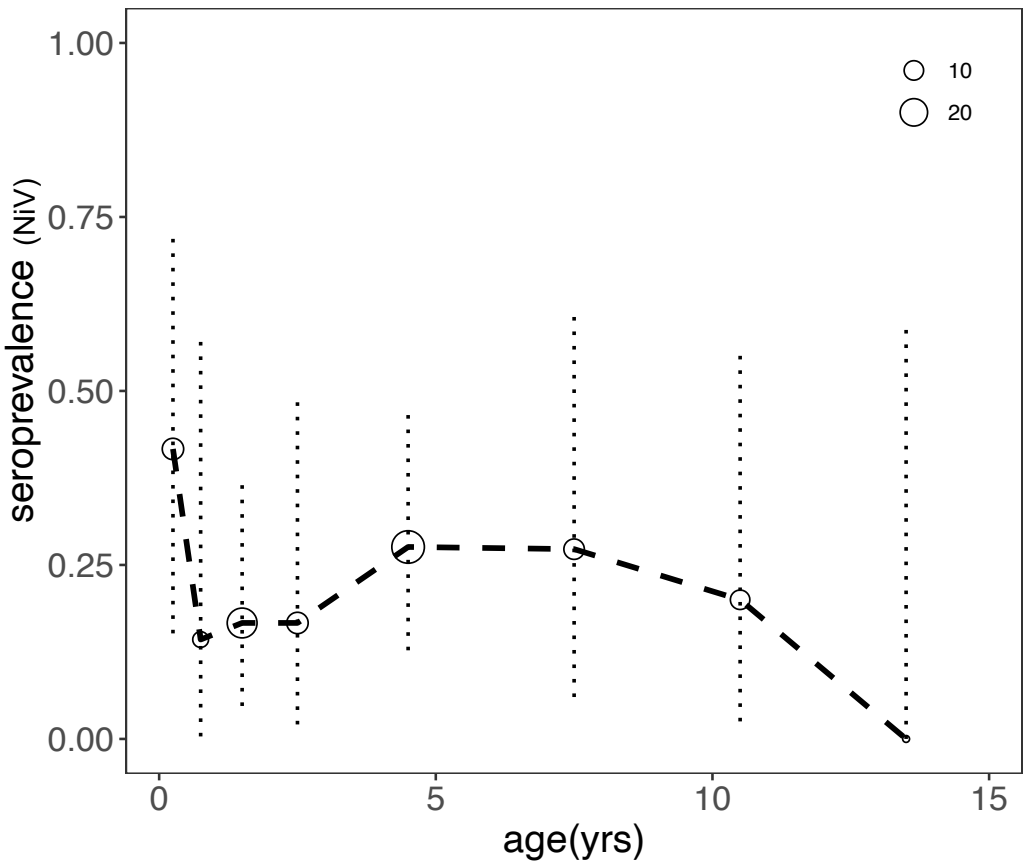
Field data show clear evidence of **seasonal cycles** of **henipaviruses** in Malagasy fruit bats.



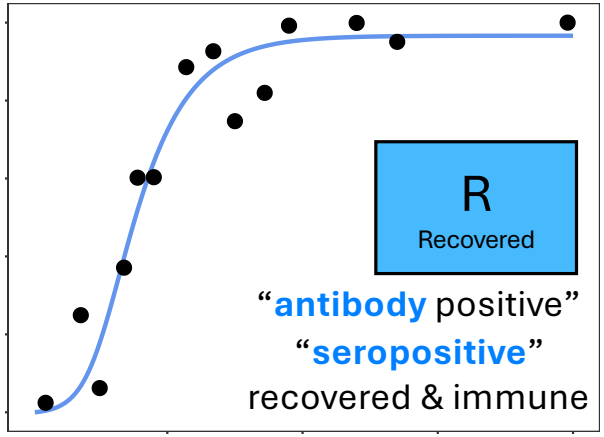
HNV serology, *E. dupreanum* bats

Step 3:

Collect data ~



Brook et al. 2019 *J of Animal Ecology*



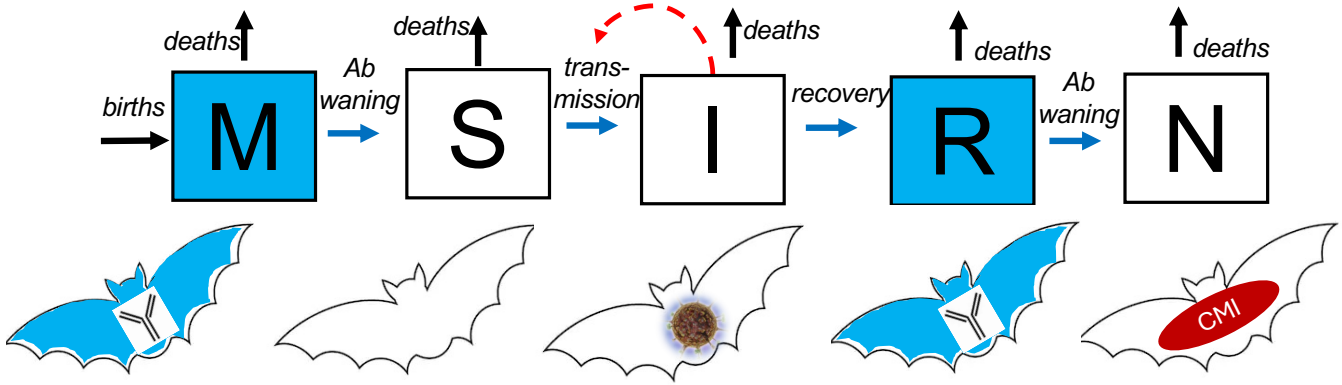
We build **age-seroprevalence** curves to infer infection dynamics.

E. dupreanum
anti-NiV serology

Step 4: Construct a model that demonstrates your hypothesis ~

Hypothesis:

Seasonal birth pulses *drive* ***zoonotic virus cycles*** *in Madagascar fruit bats.*

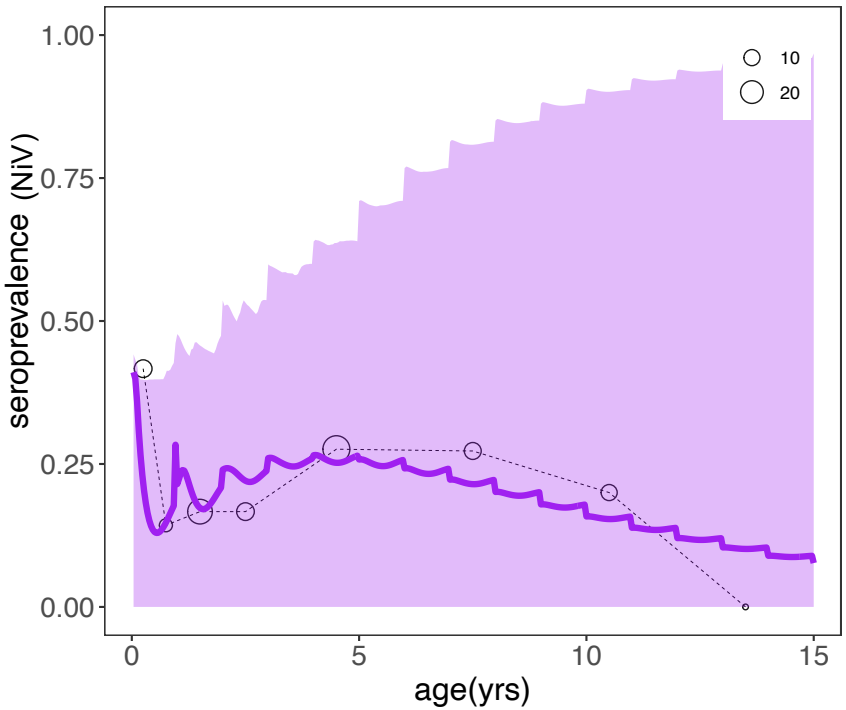


Prior work finds support for a model of **persistent immunity with waning antibodies** as the best explanation for our field data.

Brook et al. 2019 *J of Animal Ecology*

Step 5: Assess model fit to data~

The **MSIRN** model captures the broad **age-seroprevalence** profile in our original dataset.



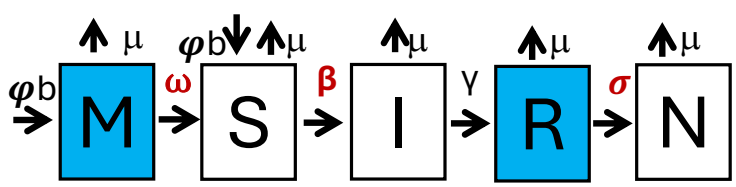
Brook et al. 2019 *J of Animal Ecology*

Parameters from first model fit to data:

ω = 0.12 biwk^{-1}
(**4 mon. maternal immunity**)

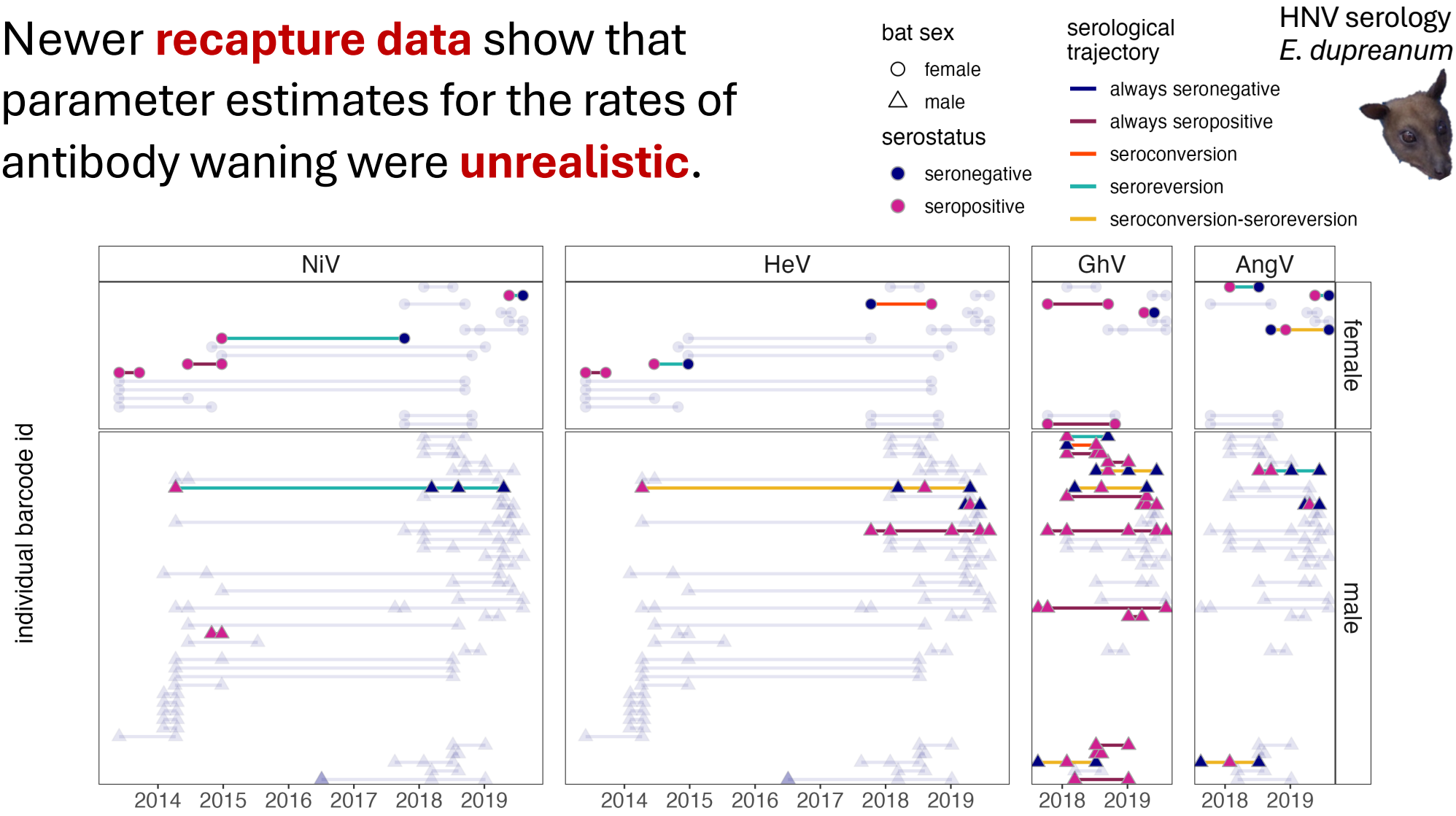
σ = .01 biwk^{-1}
(**3.85-yr duration AB-mediated immunity**)

β = 1.96 infectious contacts per capita biwk^{-1}



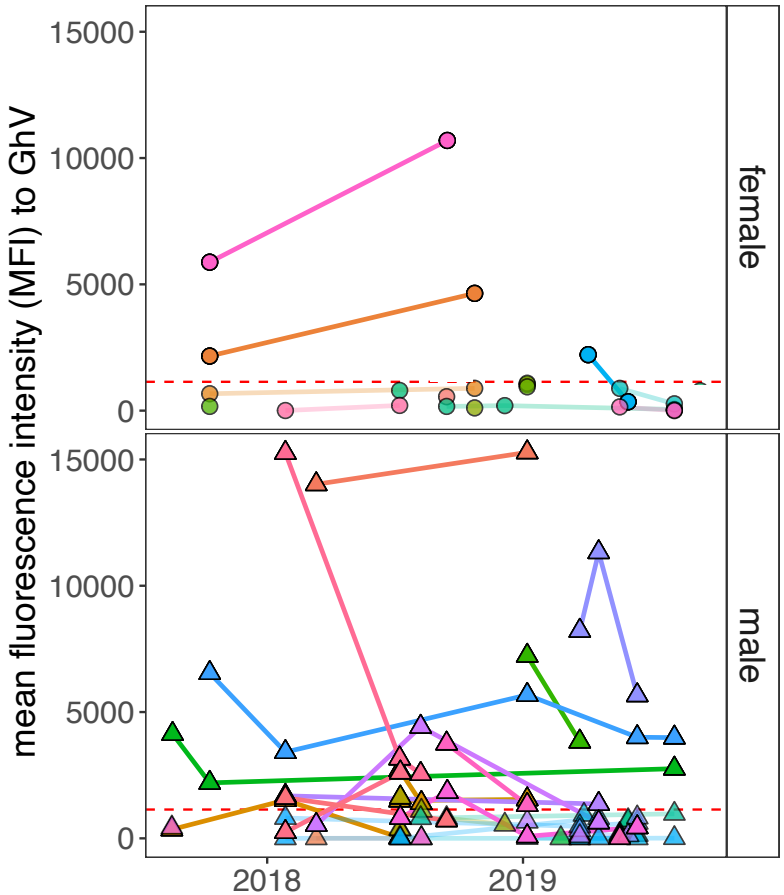
E. dupreanum
anti-NiV serology

Newer **recapture data** show that parameter estimates for the rates of antibody waning were **unrealistic**.



We can examine **quantitative measures of antibody binding** (MFI) through time in recaptured bats.

GhV serology
E. dupreanum



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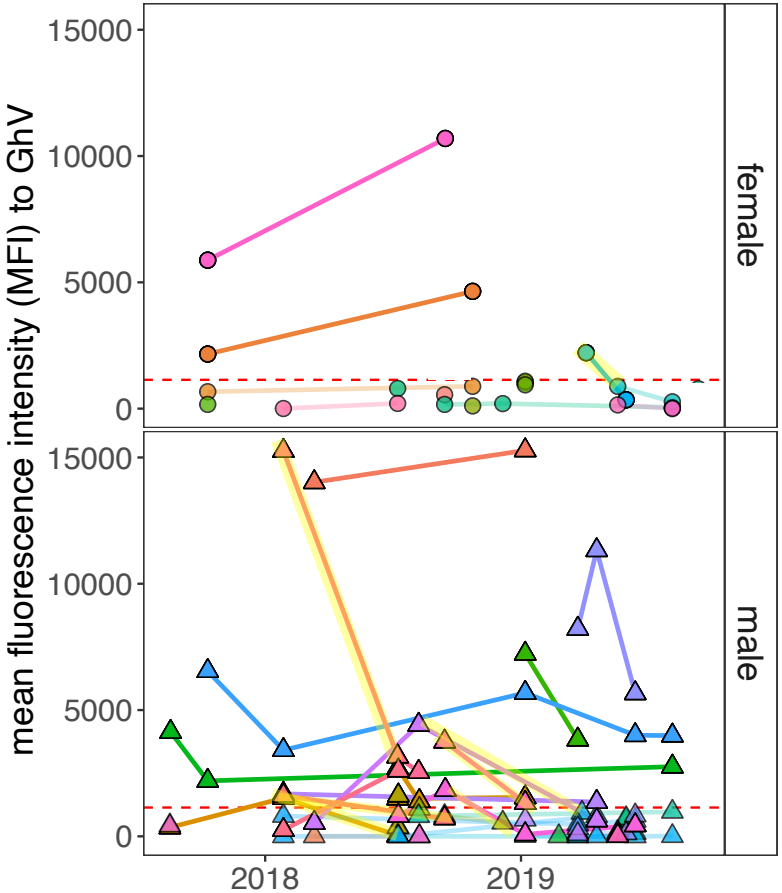
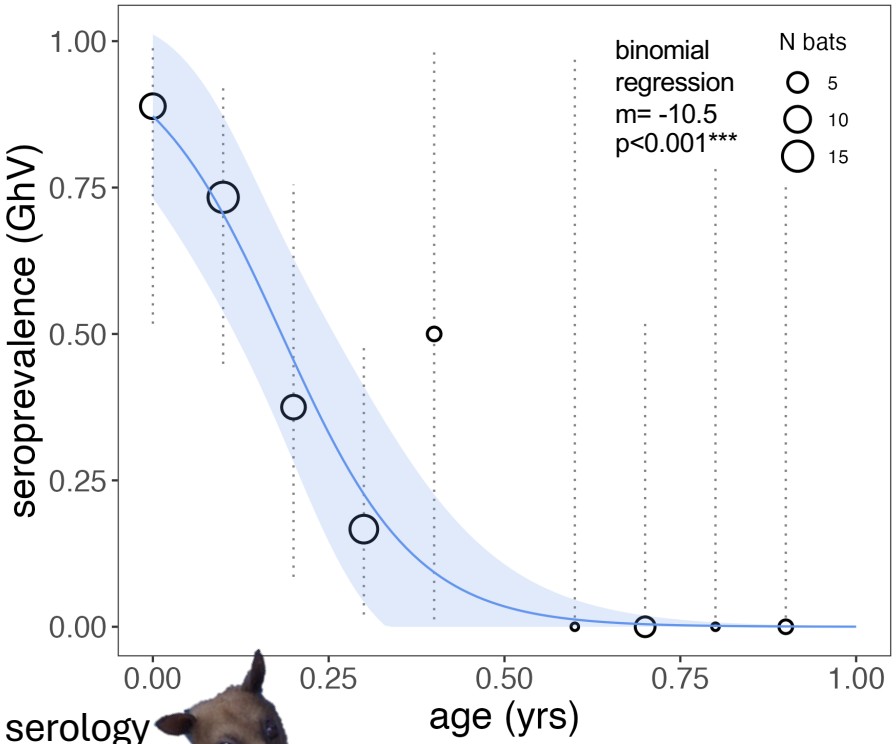
GhV serology
E. dupreanum



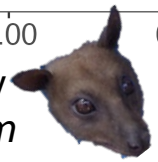
Antibody waning is **rapid**!

<1 year for most adult bats, suggesting that previous estimate of a *4-year duration of adult antibody immunity* is unrealistic.

Antibody waning is also rapid in neonates, faster than previously estimated by models fit to data.



GhV serology
E. dupreanum

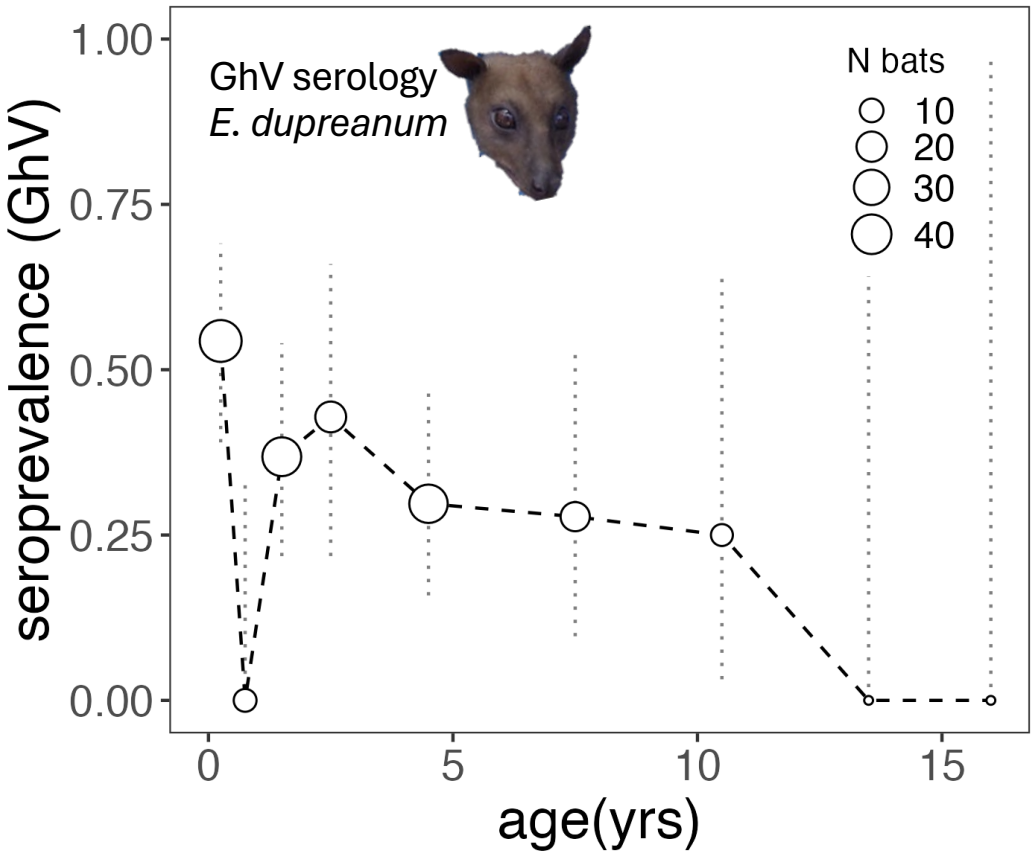


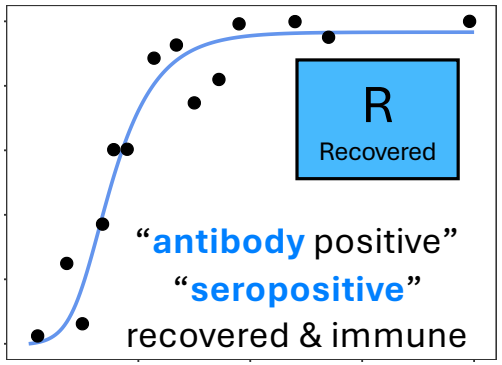
Mean duration of maternal immunity **~1.5 months**.

Population maternal immunity has fully waned by 7 months.

Malagasy flying fox pup.
Photo by Cara Brook

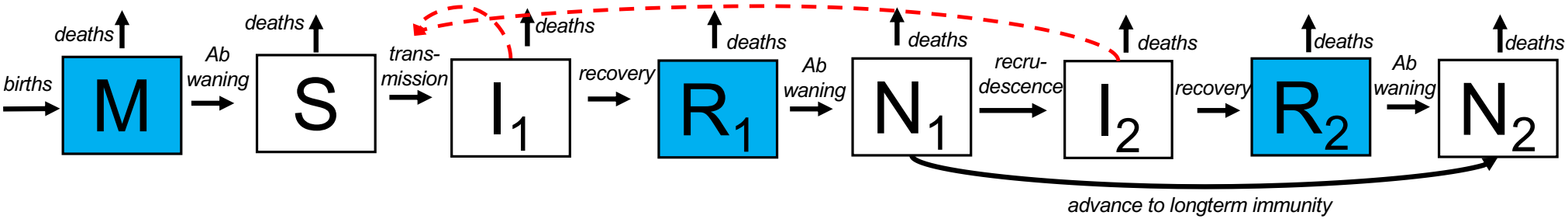
New data allows us to improve previous **age-seroprevalence** curves for henipaviruses.





Step 6: Optimize model parameters to best fit the data ~

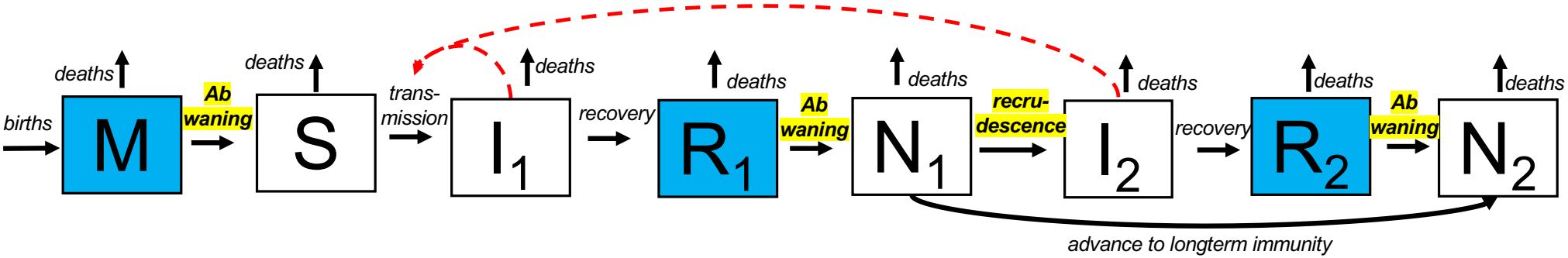
GhV serology
E. dupreanum



New data offers support for an adjusted model that incorporates **recrudescent secondary infections** in a subset of individuals.

Step 6: Optimize model parameters to best fit the data ~

GhV serology
E. dupreanum



fixed duration maternal immunity

1.5 months

constrained duration adult Ab immunity

< 1 year

estimated duration non-Ab immunity

~1 yr

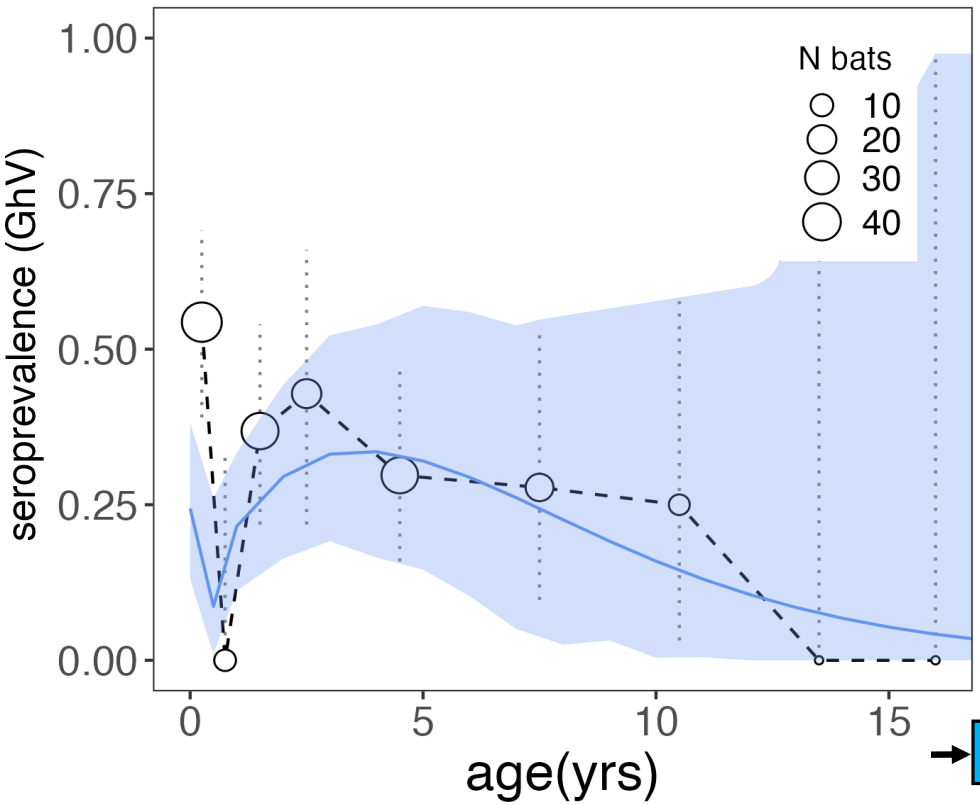
estimated duration secondary adult Ab immunity

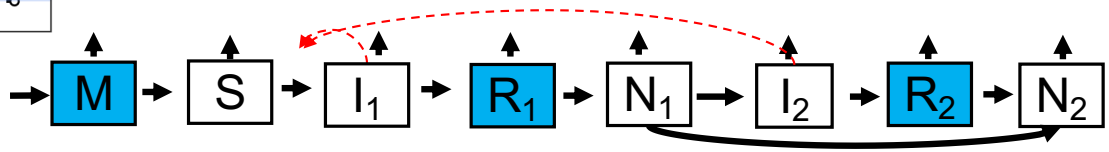
~2 yrs

Step 6: Optimize model parameters to best fit the data ~

*This model provides a **better fit** to our newer **age-seroprevalence** and recapture **data**.*

GhV serology
E. dupreanum

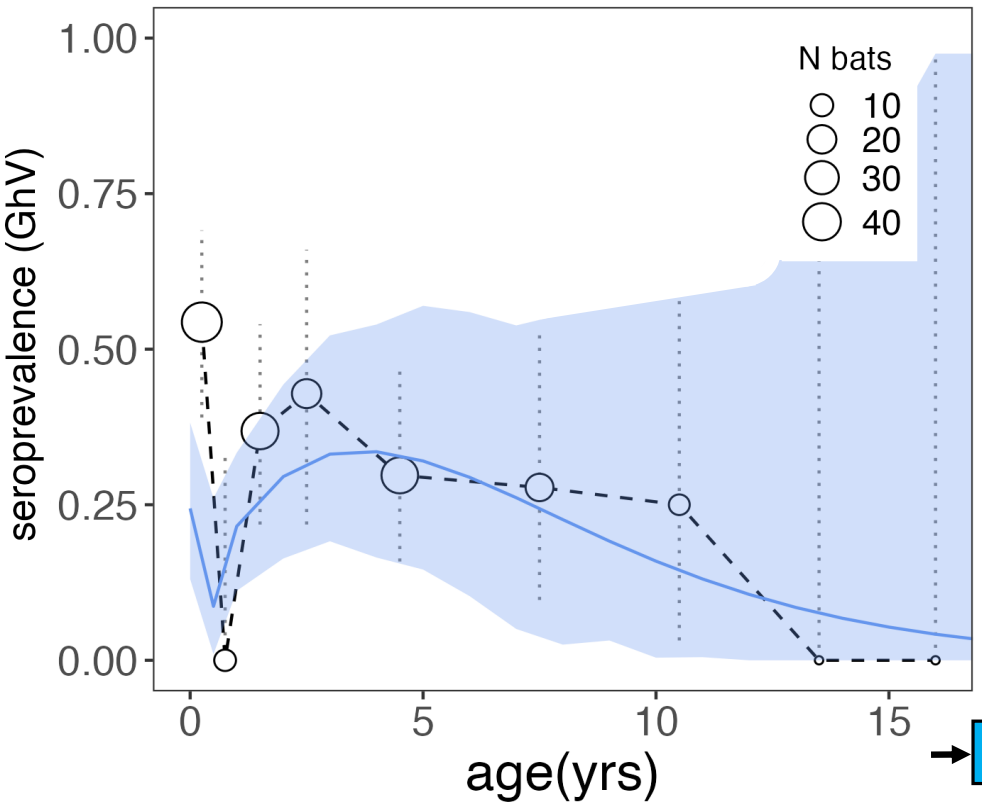




Step 6: Optimize model parameters to best fit the data ~

*This model provides a **better fit** to our newer **age-seroprevalence** and recapture **data**.*

GhV serology
E. dupreanum



Parameters from new model fit to data:

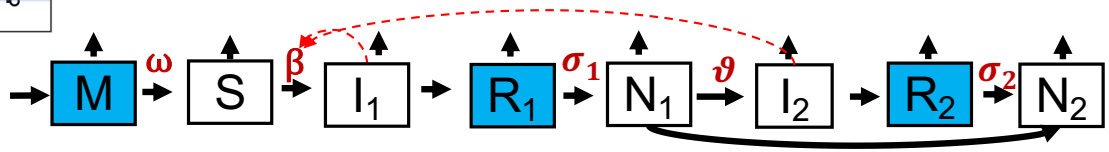
ω = 10.49 yrs^{-1}
(~1.5 mon. duration maternal immunity, fixed)

ϑ = 1.03 yrs^{-1}
(~1 year duration immunity prior to recrudescence)

σ_1 = 10.98 yrs^{-1}
(~1 month duration AB-mediated immunity)

σ_2 = 0.157 yrs^{-1}
(~6 years duration AB-mediated immunity after 2nd exposure)

β = 6.38 infectious contacts per capita biwk^{-1}



The **bat virus literature** has debated **recrudescence** for over a decade.

Epidemiol. Infect. (2011), **139**, 1570–1579. © Cambridge University Press 2011
doi:10.1017/S0950268811000550

Evidence for Nipah virus recrudescence and serological patterns of captive *Pteropus vampyrus*

A. R. SOHAYATI[1], L. HASSAN[2]*, S. H. SHARIFAH[3], K. LAZARUS[4], C. M. ZAINI[1], J. H. EPSTEIN[5], N. SHAMSYUL NAIM[1], H. E. FIELD[6], S. S. ARSHAD[2], J. ABDUL AZIZ[7], P. DASZAK[5] AND EcoHealth Alliance[5]†

PLOS | NEGLECTED TROPICAL DISEASES

Published: August 4, 2016

Transmission or Within-Host Dynamics Driving Pulses of Zoonotic Viruses in Reservoir–Host Populations

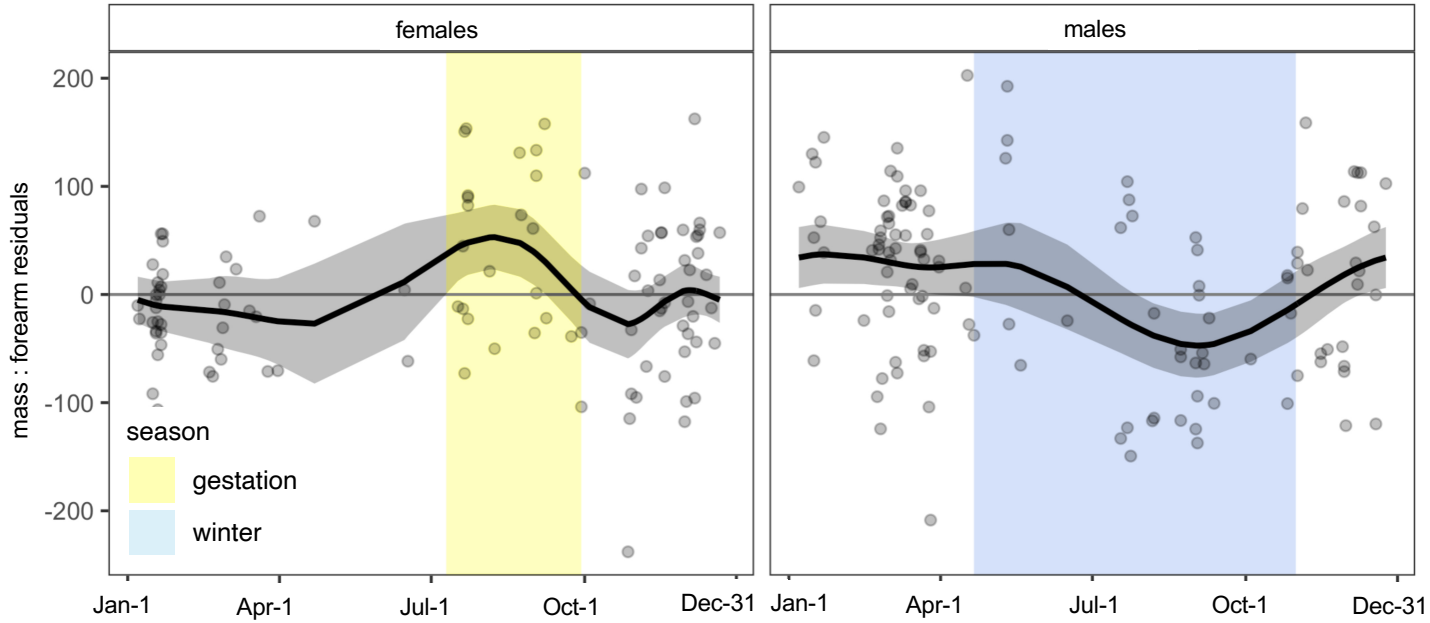
Raina K. Plowright[1,2]*, Alison J. Peel[3], Daniel G. Streicker[4,5], Amy T. Gilbert[6], Hamish McCallum[7], James Wood[8], Michelle L. Baker[9], Olivier Restif[8]

PIT tagging *R. madgascariensis*.
Photo by Cara Brook

We need **sustained monitoring of individual bats in the wild** to evaluate the hypothesis.

Correlated virus dynamics and metrics of **bat health** suggest a possible **nutritional mechanism** for **recrudescence**.

Correlated virus dynamics and metrics of **bat health** suggest a possible **nutritional mechanism** for **recrudescence**.



Male fruit bats have **lower mass** during seasonal resource scarcity (**winter**).

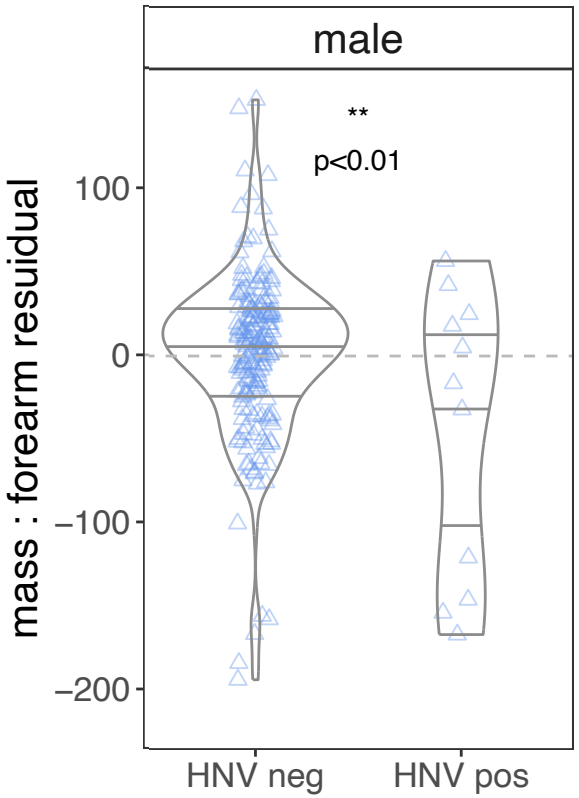
Santino Andry

Angelo Andrianiaina

PhD students, University of Antananarivo

Andrianiaina* & Andry* et al. 2022. *J Mammalogy.*

Correlated virus dynamics and metrics of **bat health** suggest a possible **nutritional mechanism** for **recrudescence**.



- We find **poorer body condition** in adult **male bats actively shedding HNVs** in urine.
- **Viral recrudescence** may be associated with **nutritional stress**.

Gwen Kettenburg,
UChicago PhD student

Step 1: Define your research question ~

*What **drives** seasonal **henipavirus** cycles in **wild fruit bats** in Madagascar?*

Step 2: Formulate a hypothesis ~

***Seasonal birth pulses** drive **henipavirus cycles** in Madagascar fruit bats.*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

Step 1: Define your research question ~

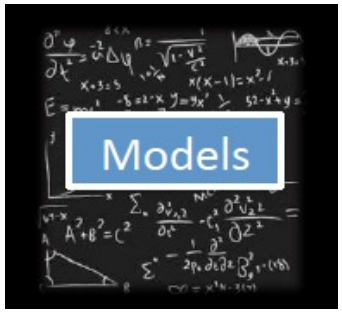
*What **drives** seasonal **henipavirus** cycles in **wild fruit bats** in Madagascar?*

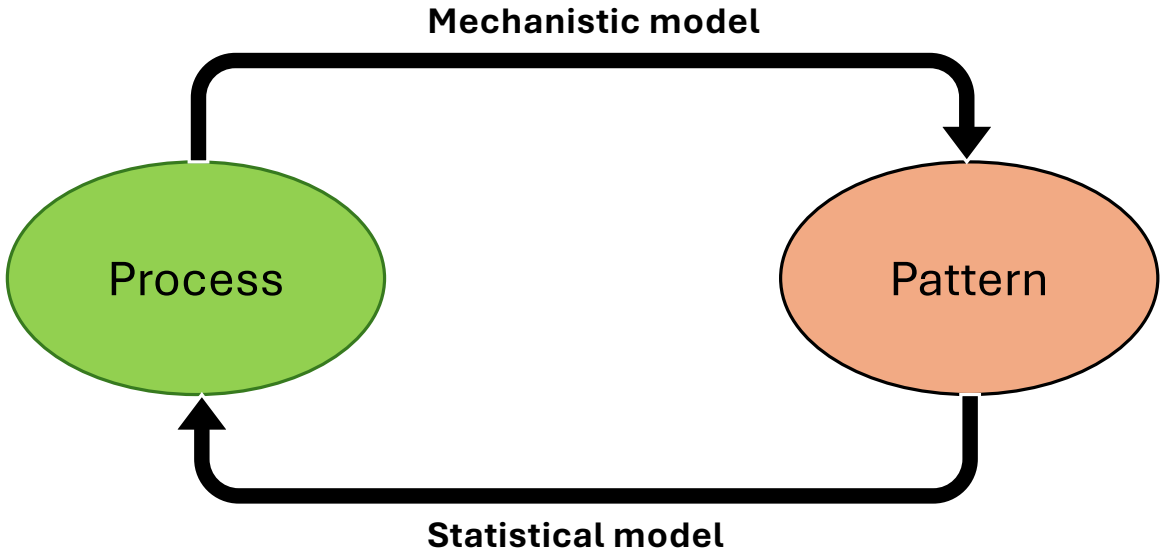
Step 7: Edit hypothesis after evaluating model fit to data ~

***Seasonal birth pulses** and **seasonal viral recrudescence from poor nutrition** drive **henipavirus cycles** in Madagascar fruit bats.*

Malagasy flying fox, *Pteropus rufus*.
Photo by Samantha Kreling.

It is ideal when statistical and mechanistic models meet:





Misaotra betsaka!

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Misaotra betsaka!

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