

SPEEDIER: Genomic/genetic Sequencing

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November 2018

IBAHCM, University of Glasgow

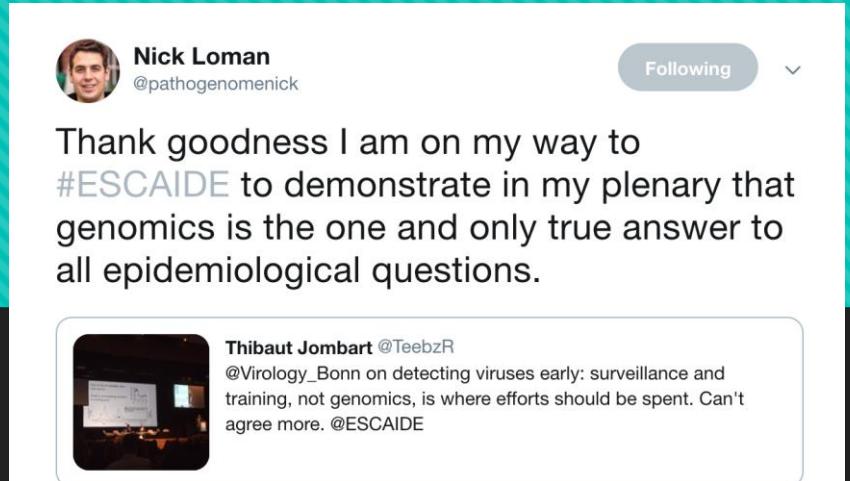
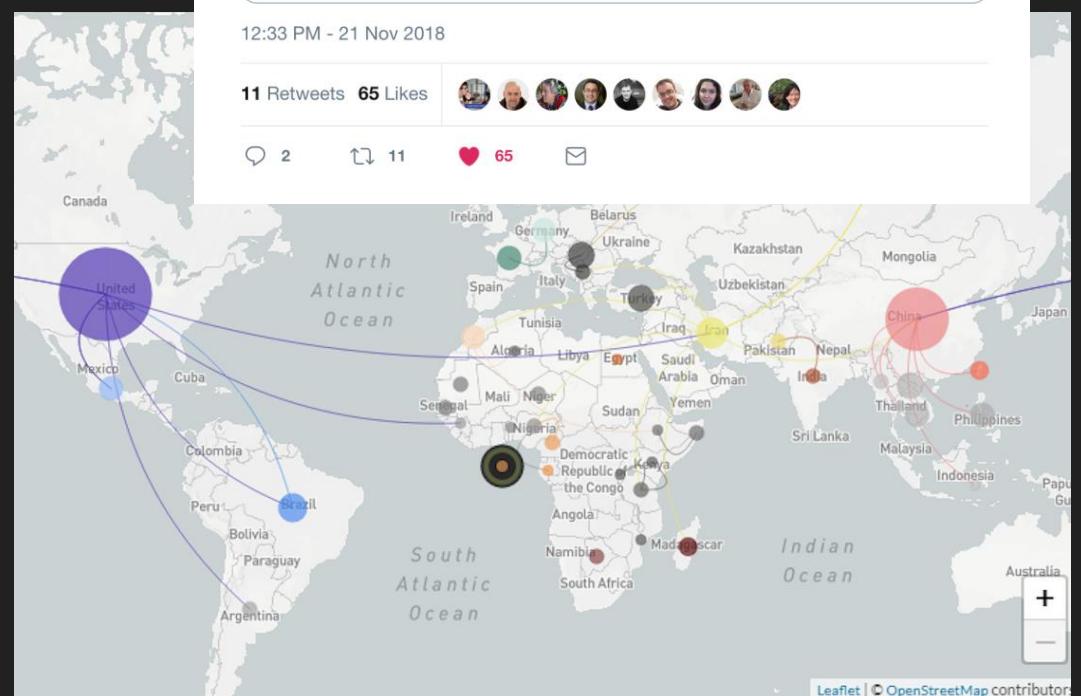


Outline

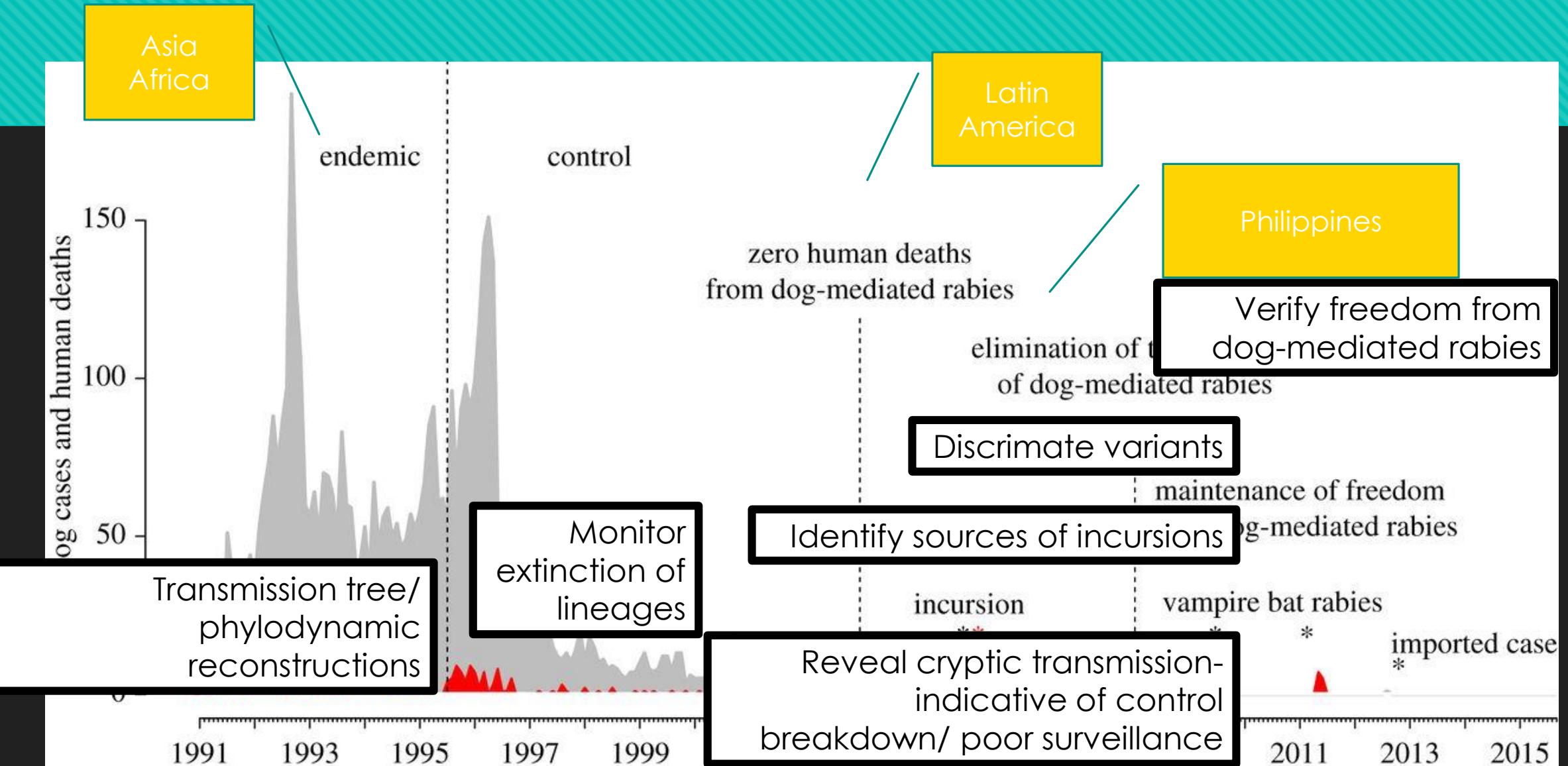
- Why do genomic surveillance?
 - Examples
- How do we do it?
 - Lab pipeline
 - Bioinformatics
 - Analysis & communication (GLUE: Josh/Rob)
- Run-through of Philippines data

Genomic surveillance

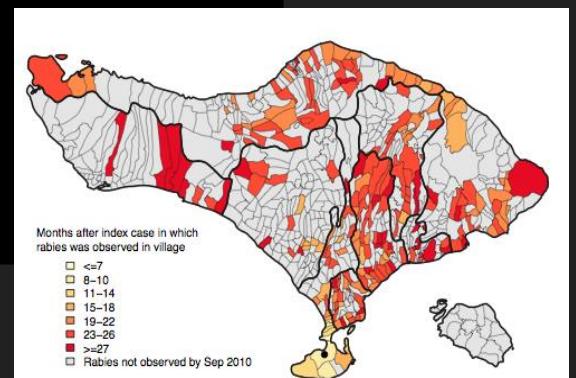
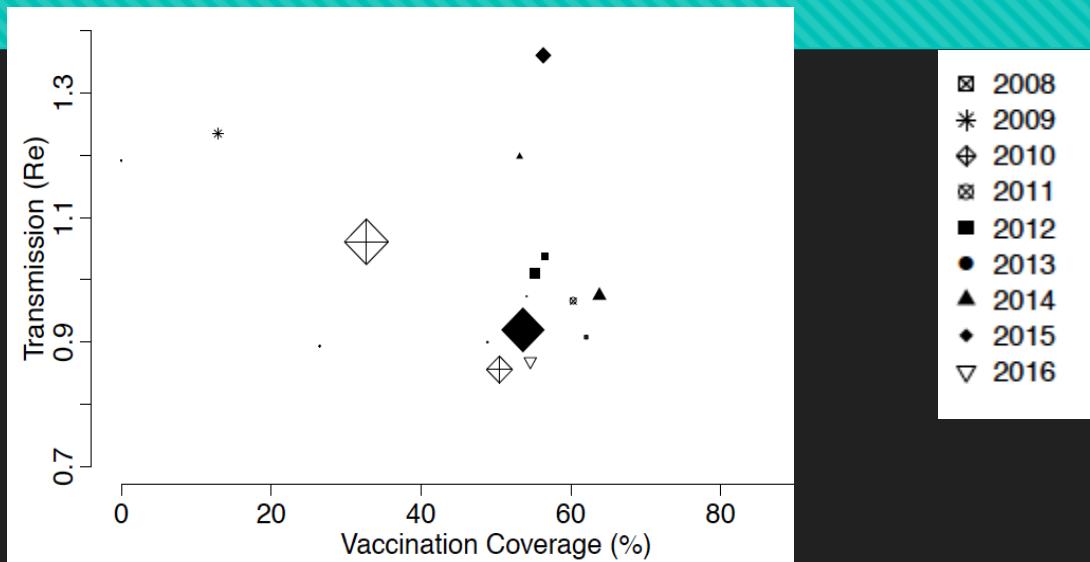
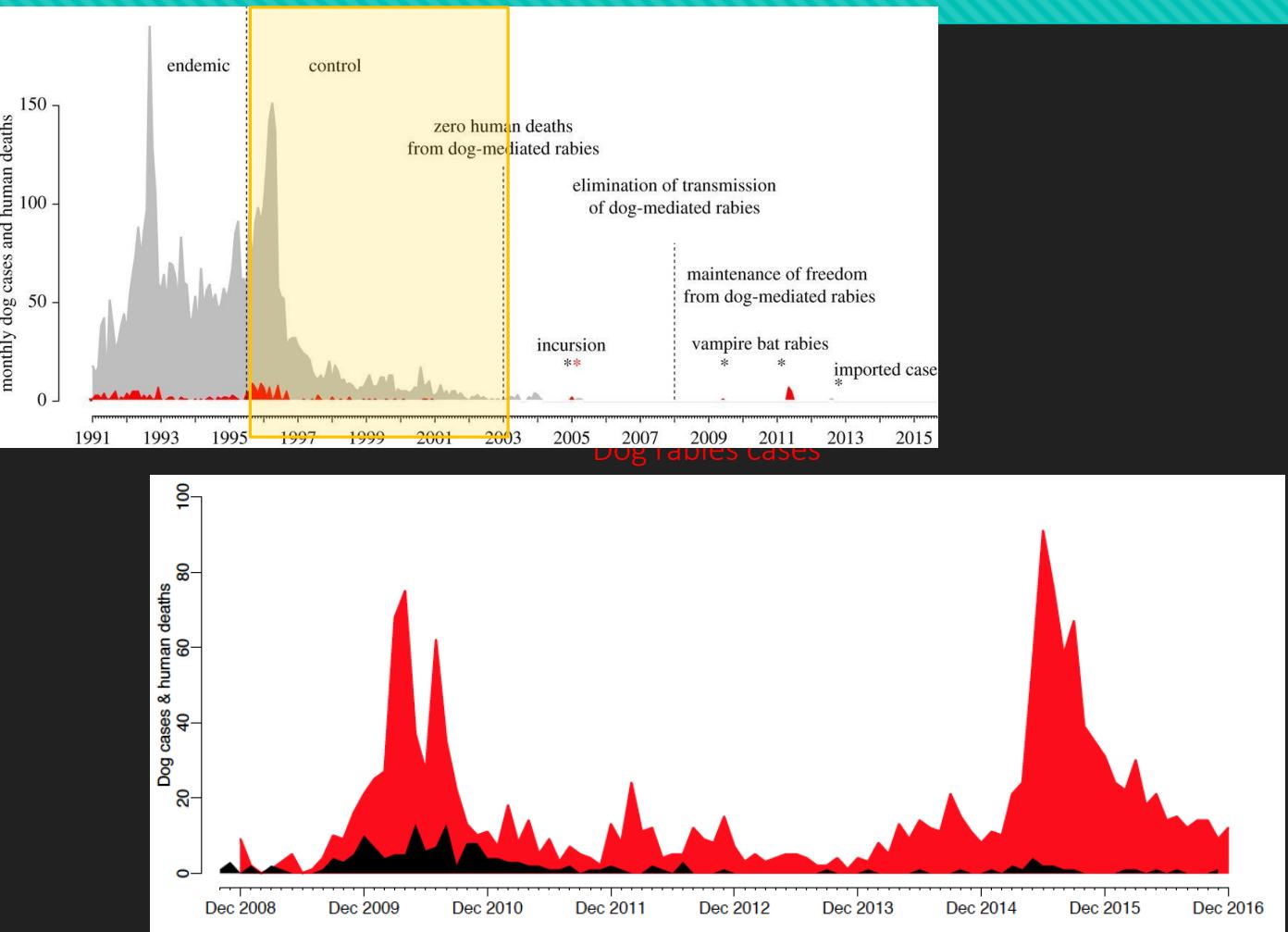
- Genomic data is emerging as a new and powerful surveillance tool
 - Insights into pathogen evolution, dynamics and spread
 - Provide actionable data
 - More achievable due to new technologies



Road to elimination



Genomic Surveillance may identify vaccine issues Bali, Indonesia



Genomic surveillance can distinguish wildlife variants, cryptic transmission & incursions



250 km
~400,000 dogs

Mexico, Latin America rabies control

Rabies maintained locally in dog population or seeded by importations?

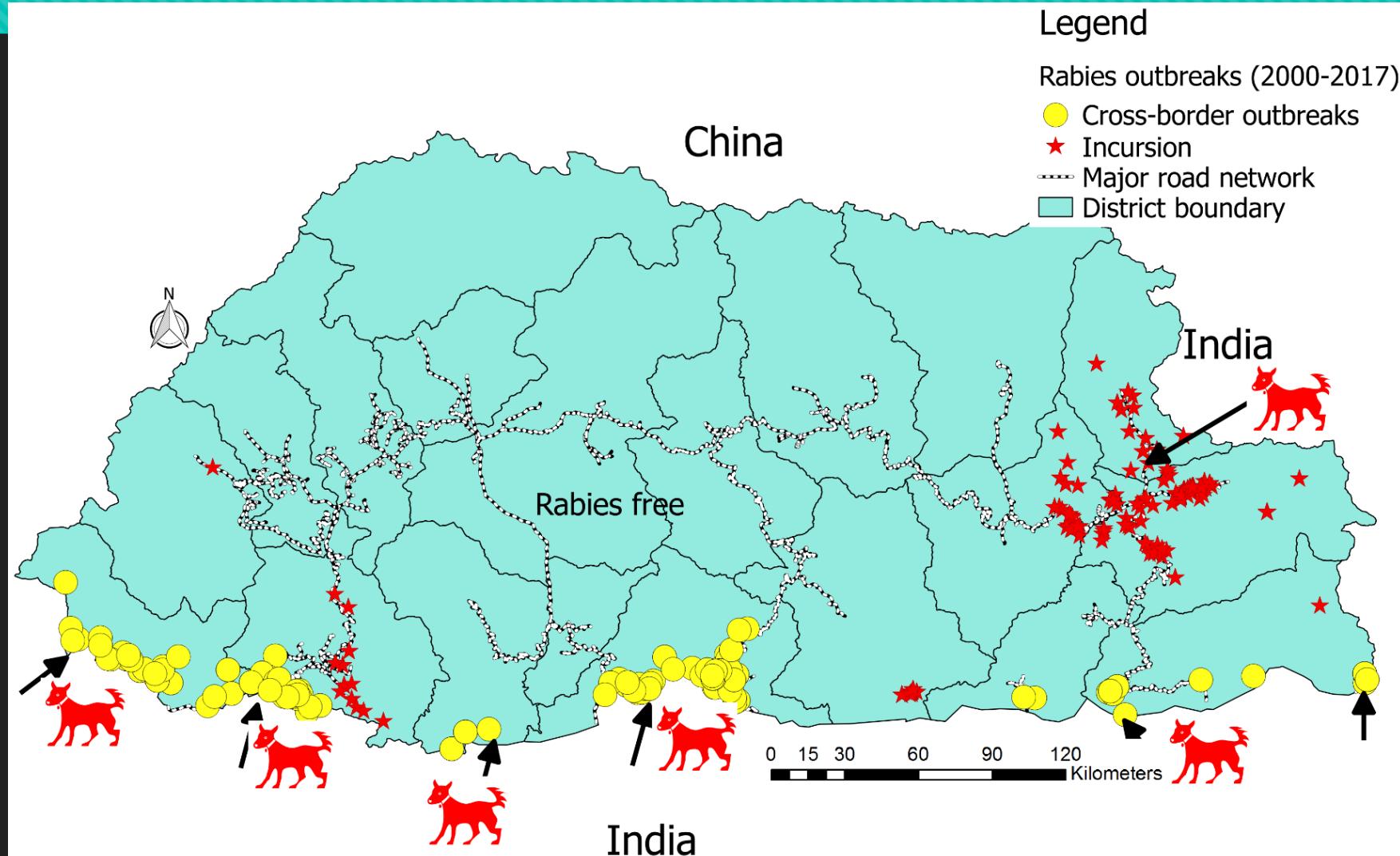


Cross-border transmissions: a case study from Bhutan and India



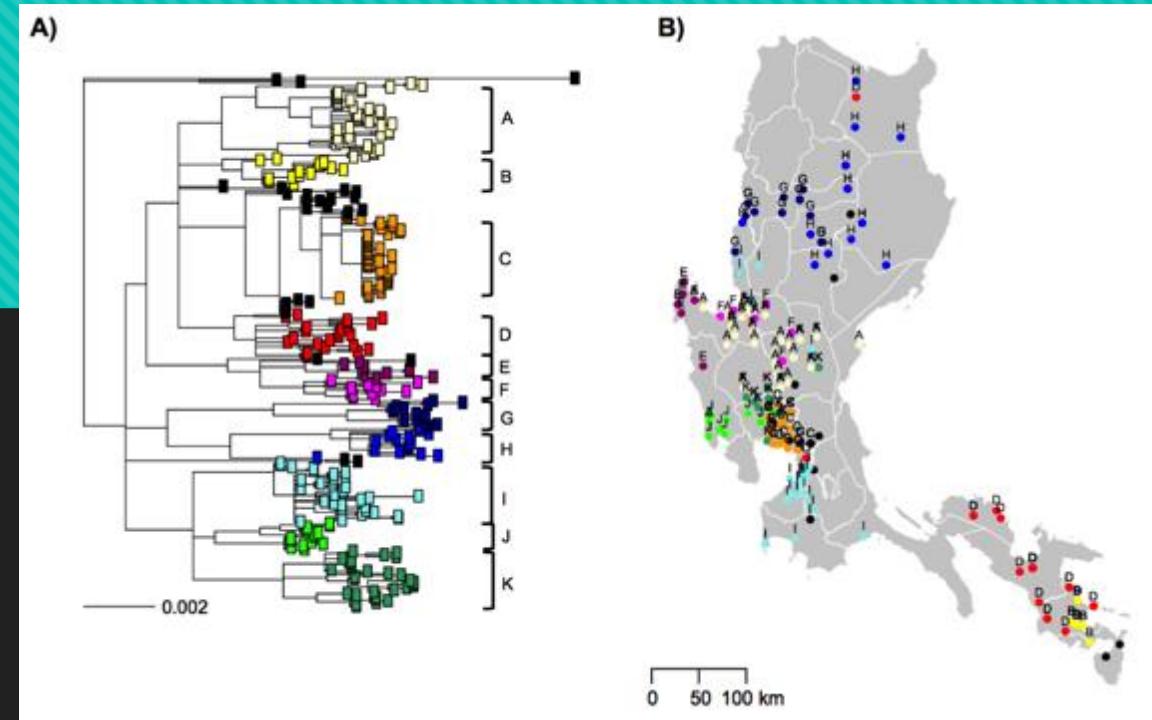
Cross-border
transmission and
incursions -
“Endgame” challenges
for rabies freedom!

Tenzin et al. 2008, 2009, 2010,
2011, 2012, 2015, 2016, 2017

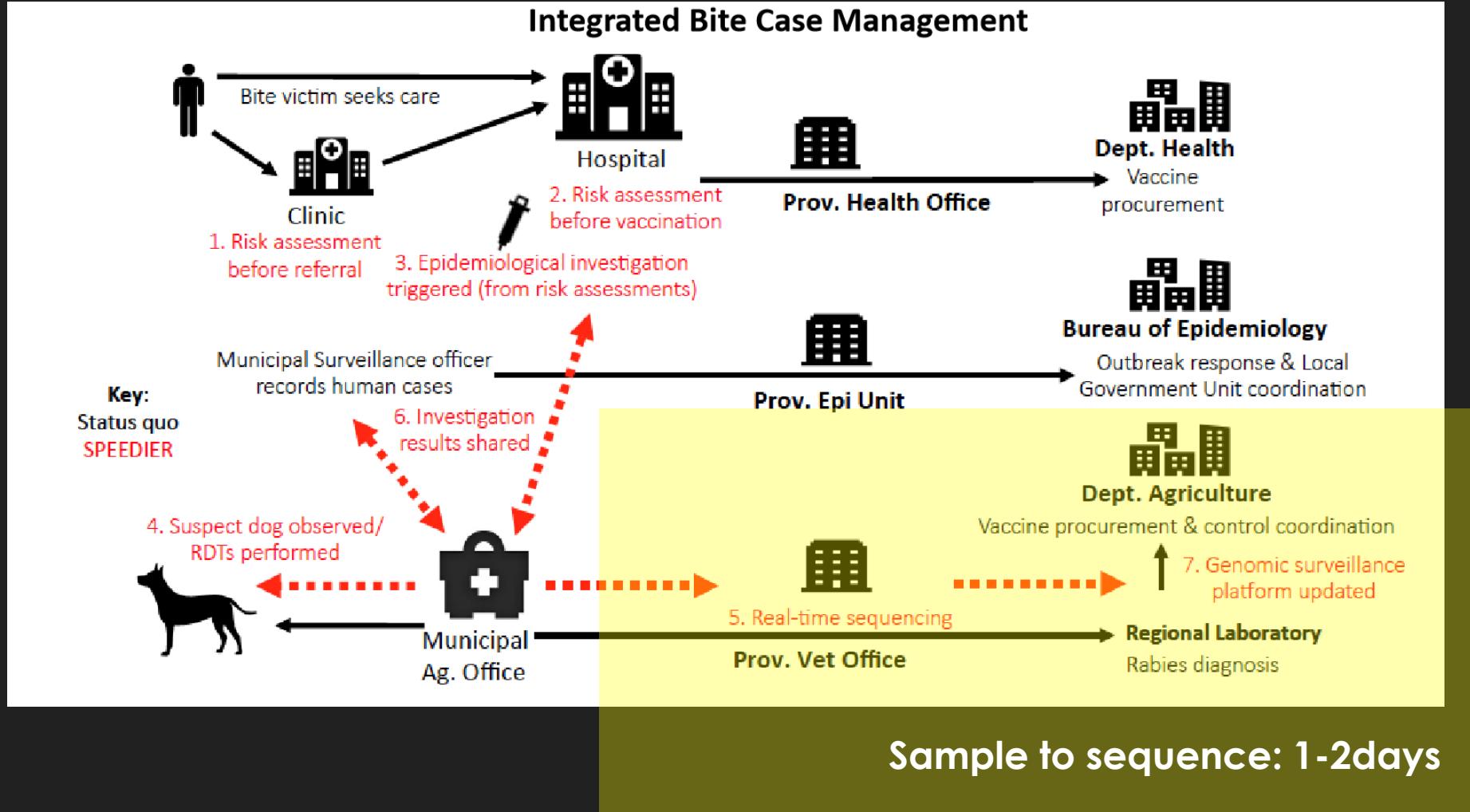


Philippines

- Spatial scale
- Island dynamic
- Differentiating between inter and intra-island transmission dynamics
- Incursions v cryptic transmission
- Factors driving these processes



Integrated Bite Case Management



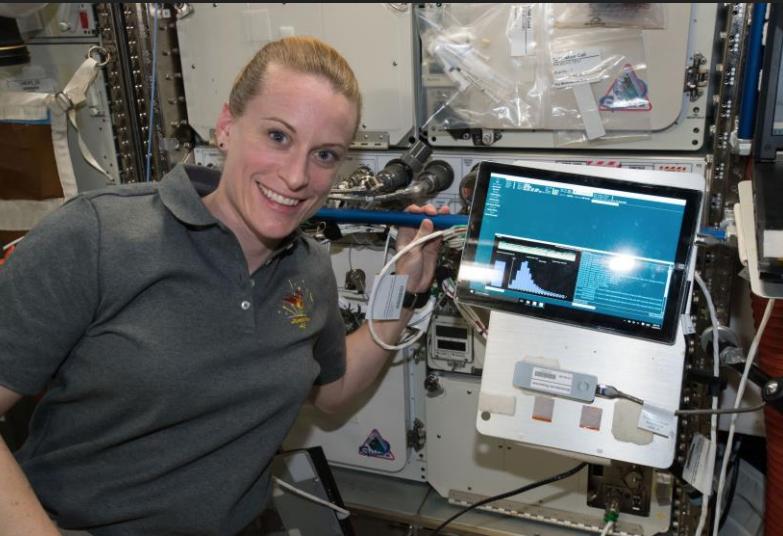
The MinION

- a portable DNA sequencer



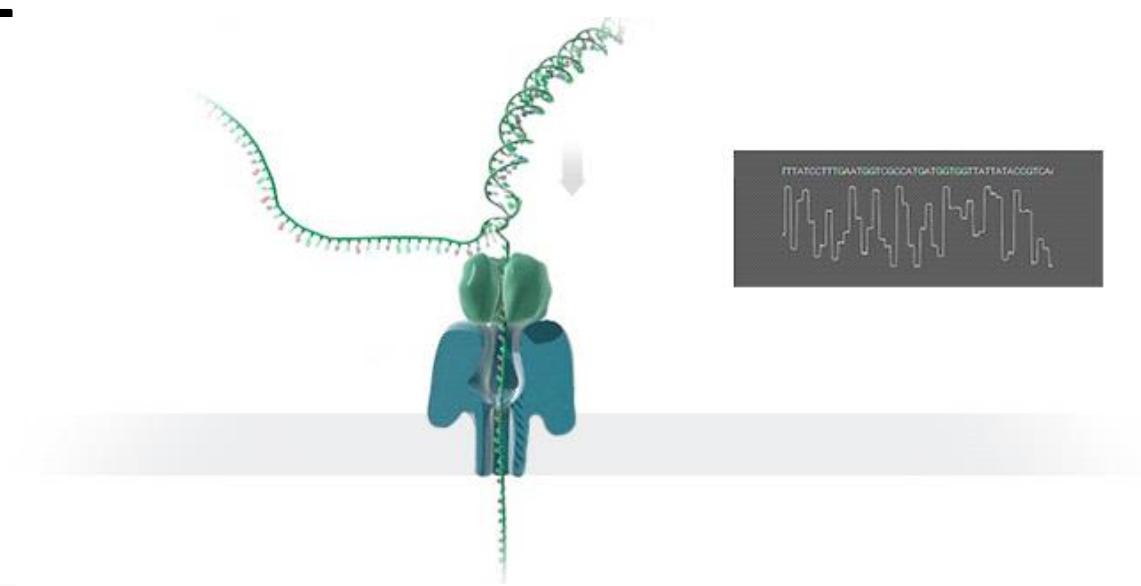
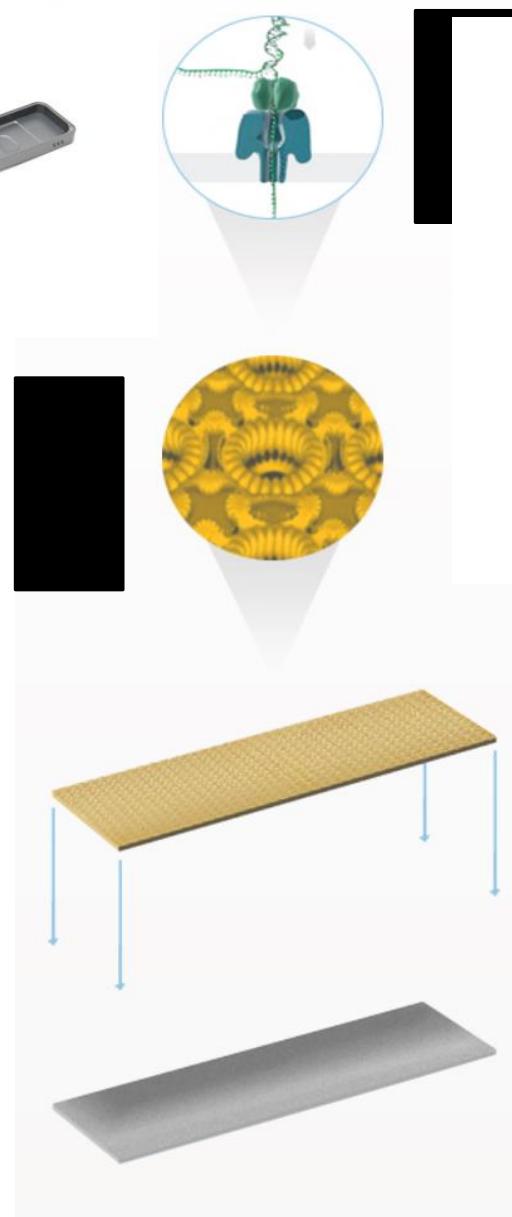
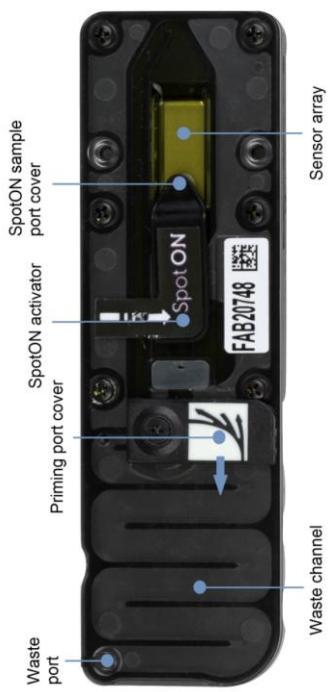
- Portable
- Real-time (Run until... sufficient data)
- Capable of ultra-long reads
 - Current record=2.3million base pair read
- Simple workflows
- Affordable- no capital cost
- Scalable technology
- Laptop powered
- Direct DNA/RNA sequencing
- Exciting developments





"Democratic sequencing" – anywhere, anytime, anyone





RNA EXTRACTION

- Brain tissue
- FTA card

ENRICHMENT

- "Primal seq"
- Multiplex PCR

Quick et al
(2016), Nat
Protoc

NGS LIBRARY

- Oxford Nanopore
Kits



MinION SEQUENCING

BIOINFORMATICS

- Lab-on-a-ssd



ANALYSIS

- Publically
available RABV-
GLUE



REPORT

- Translate
results to end
users



ARTICnetwork

Oxford
NANOPORE
Technologies

Lab in a suitcase

- Outbreaks often occur in settings with minimal laboratory capacity
- Portable platform capable of *in situ* outbreak surveillance could help provide rapid epidemiological information relevant to control

Mobile sequencing on a medical road trip to track Zika spread

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8 JUN 16



Liz Harley



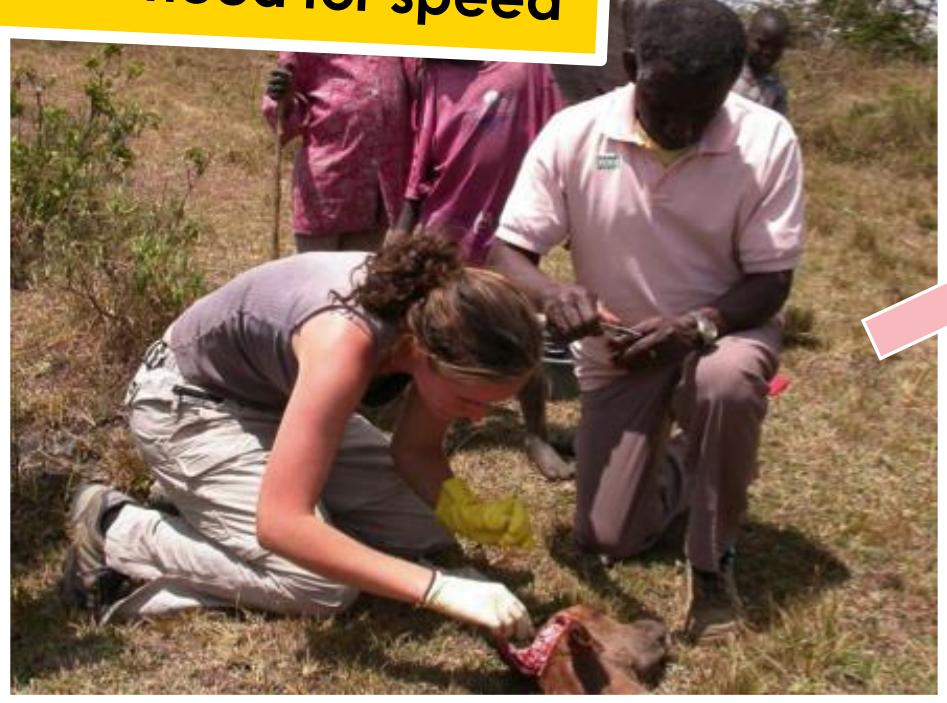
Mobile DNA sequencing in Joao Pessoa, Brazil. Image by Ricardo Funari

From Ebola to Zika, tiny mobile lab gives real-time DNA data on outbreaks

A genomic surveillance system which fits in a suitcase can help health workers to quickly understand the spread of viruses and break the chain of infection

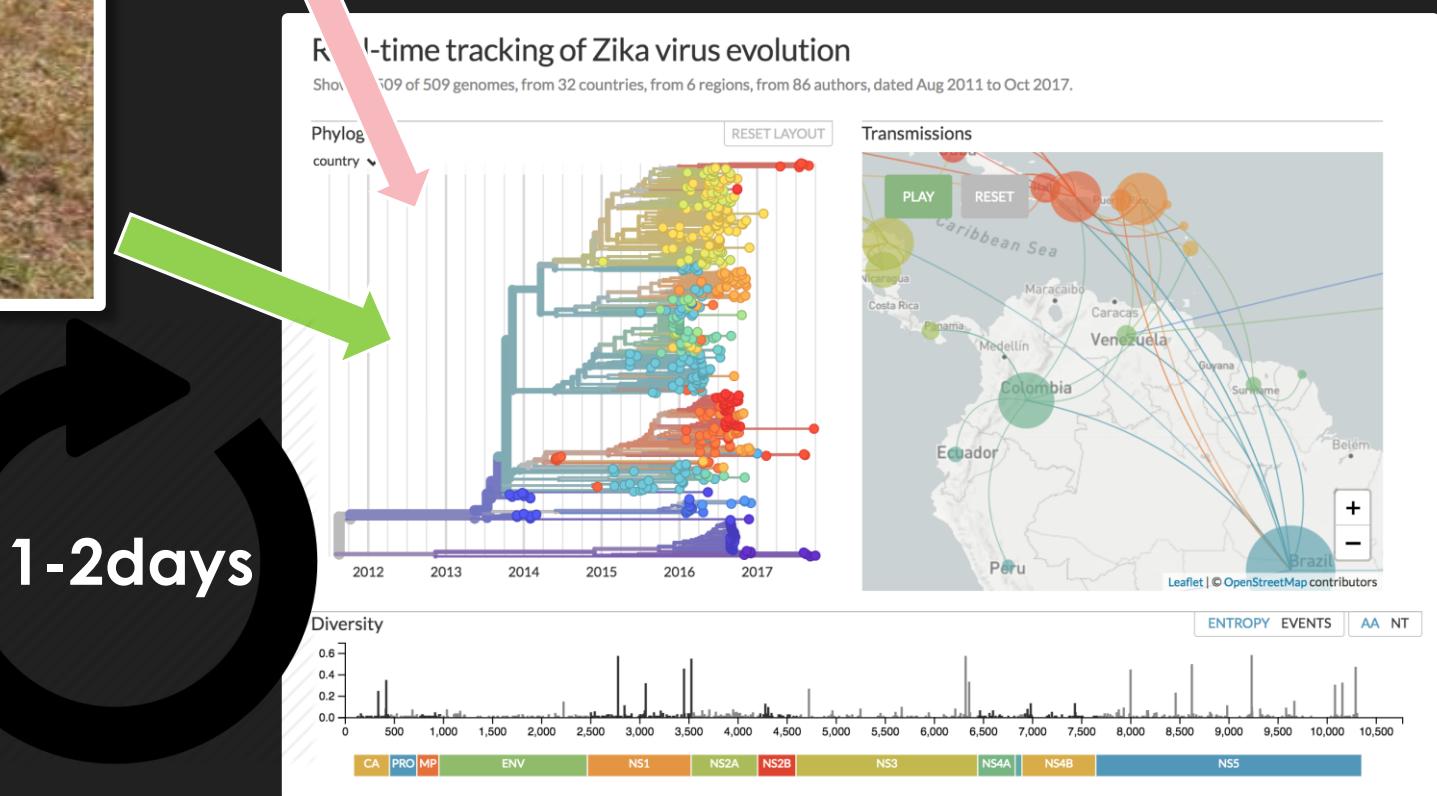


GETTING THE DATA -and the need for speed



3-5months

Shipment to UK
rabies
reference lab



**KEEP
IT LOCAL**

1-2days

Putting together a (sequencing) lab in a suitcase

- Portability
- Power supply
- Cold chain
- Contamination
- Consumables

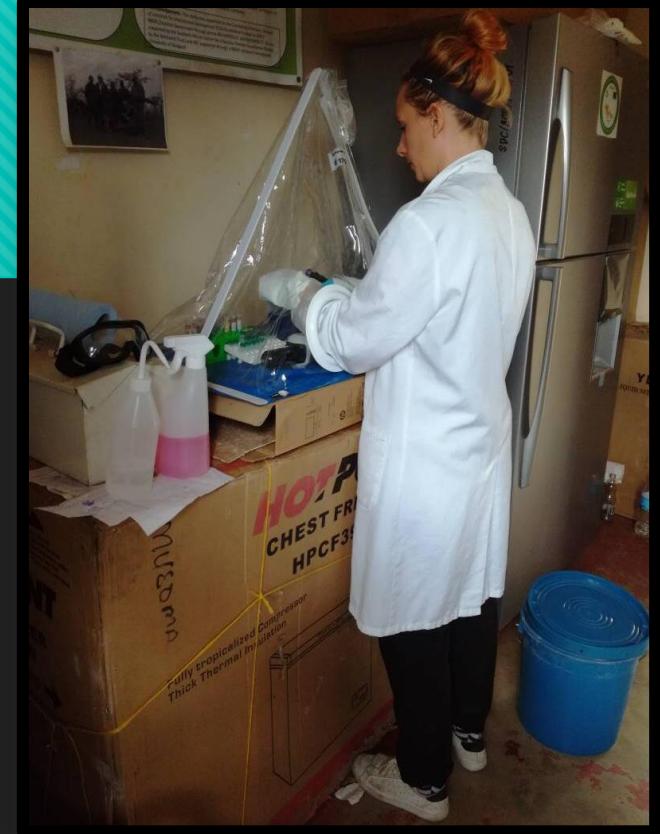




RNA extractions

- Brain tissue homogenized in RNA shield
 - Inactivates virus
 - Virkon & UV to decontaminate surfaces

- Quick RNA miniprep kit from Zymo Research
 - Column-based i.e. centrifuge steps



Biosafety

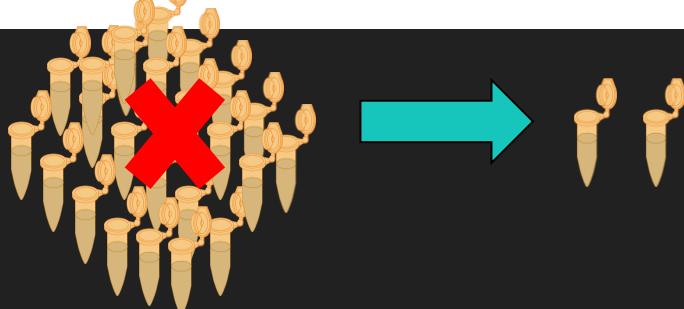
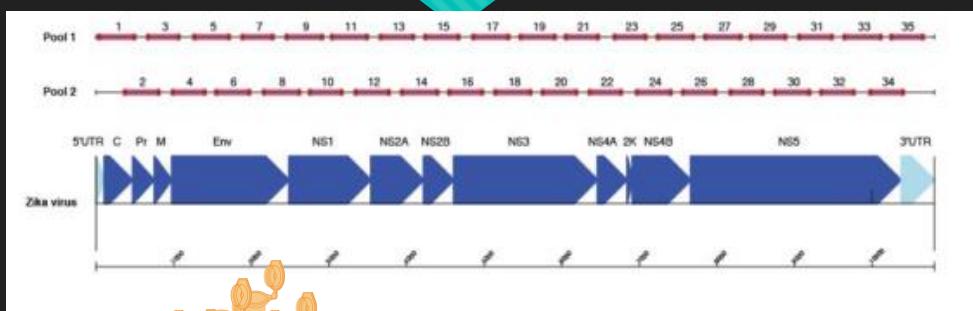
UK=CL3

Tanzania=Glove box

Philippines=CL2?

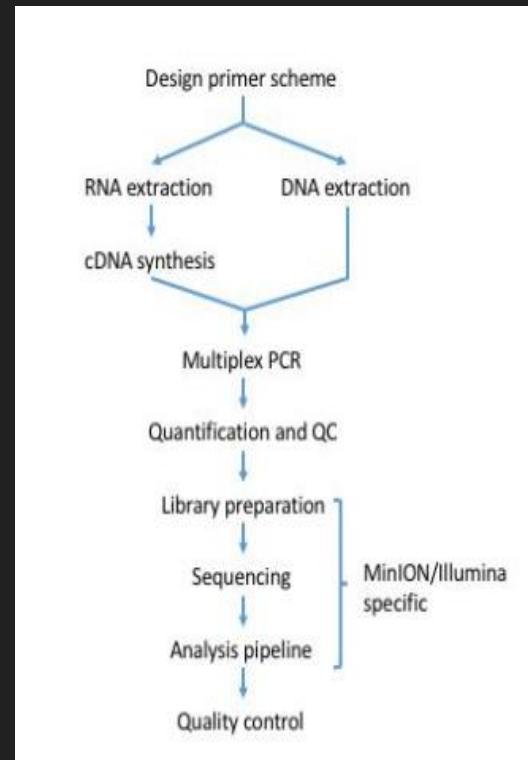
Viral enrichment

Multiplex PCR



Philippines: Will need to design primers

End to End Protocol Quick et al (2017), Nature Protocols



TIMING

- Steps 1-2, Design and ordering of primers: 1 h
- Steps 3-4, RNA extraction and QC: 1 h
- Steps 5-9, Preparation of cDNA: 1 h
- Steps 10-11, Preparing the primer pools: 1 h
- Steps 12-14, Performing multiplex tiling PCR: 5 h
- Steps 15-17, Cleanup and quantification of amplicons: 1 h
- Step 18, Library preparation and sequencing: 1-2 d
- Steps 19-23, Data analysis: 1-2 h
- Steps 24-25, Quality control of consensus sequences: 1 h

DNA library for sequencing

MinION

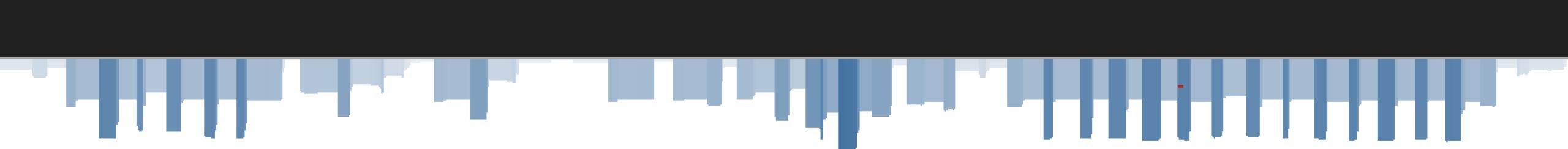
- ONT sample prep: modified protocols
- Current protocol= up to 12 samples/run
- Future protocol= up to 96 samples/run



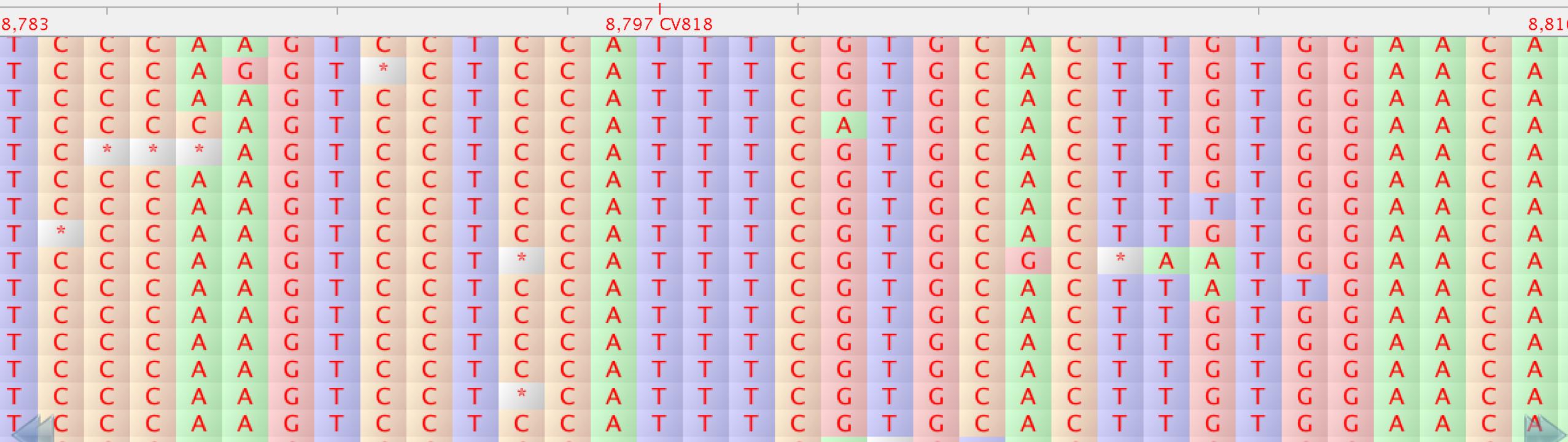
Illumina

- Nextera XT sample prep
- Up to 384 samples

Multiplexing
samples to reduce
costs



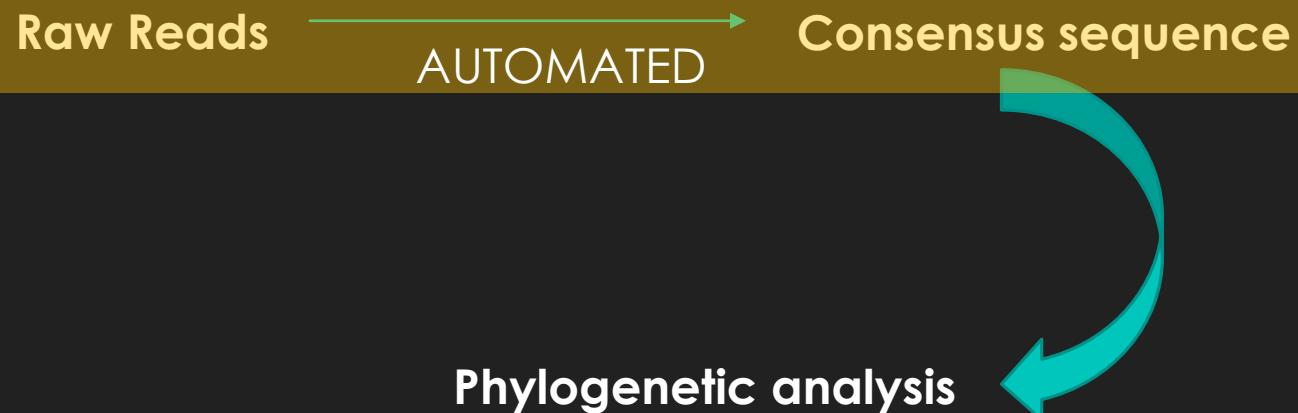
CIGAR-I
CIGAR-D
CIGAR-RIGHT-CLIP
CIGAR-LEFT-CLIP





Bioinformatics

- Aim to be as automated as possible
- Lab-on-a-ssd or local install
 - No setup or one initial install
 - Does not require bioinformatic skills
 - Follow SOP
- Data storage: SSD or servers



Kirstyn Brunker
@kirstynbrunker

Lab-on-a-ssd: the counterpart to [#labinasuitcase](#)! Field bioinformatics made easy by [@NetworkArtic](#)- even for eejits like me! [#fieldseq](#)



12:45 PM - 24 Oct 2018

10 Retweets 37 Likes



10



37



First insights and context: GLUE



Rob Gifford



Josh Singer

RABV-GLUE Home Sequence Data ▾ Analysis ▾ Offline version About ▾

RABV-GLUE

A Sequence Data Resource for Rabies Virus

Rabies virus (RABV) is the virus that attacks the nervous system, causing rabies disease. In the wild rabies virus has been found to infect many mammalian species. Rabies results in encephalitis in humans and other mammals and is almost always fatal in unvaccinated humans.

The rabies virus is the type species of the Lyssavirus genus of the Rhabdoviridae family. These viruses are enveloped and have a single stranded, negative-sense RNA genome.

RABV-GLUE is a data-centric bioinformatics resource which organises RABV genome sequence data along evolutionary lines. RABV-GLUE aims to leverage new and existing RABV sequences in order to improve our understanding of the epidemiology and pathology of RABV.

The web version of RABV-GLUE can be used for basic analysis. An offline version of the resource can be installed by bioinformaticians and used for more advanced work.

Web site highlights

- An [analysis tool](#) providing genotyping, analysis and visualisation of submitted FASTA sequences.
- A database of RABV [sequences](#) and metadata from NCBI, updated daily and arranged into major and minor [clades](#).
- Pre-built multiple-sequence alignments of NCBI sequences, which may be downloaded in user-defined sections. Use the [clade tree](#) to select a clade such as [Africa-2](#). Then under the Download button select "Alignment".

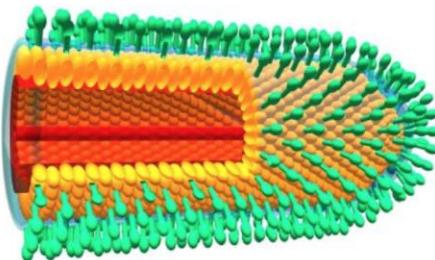
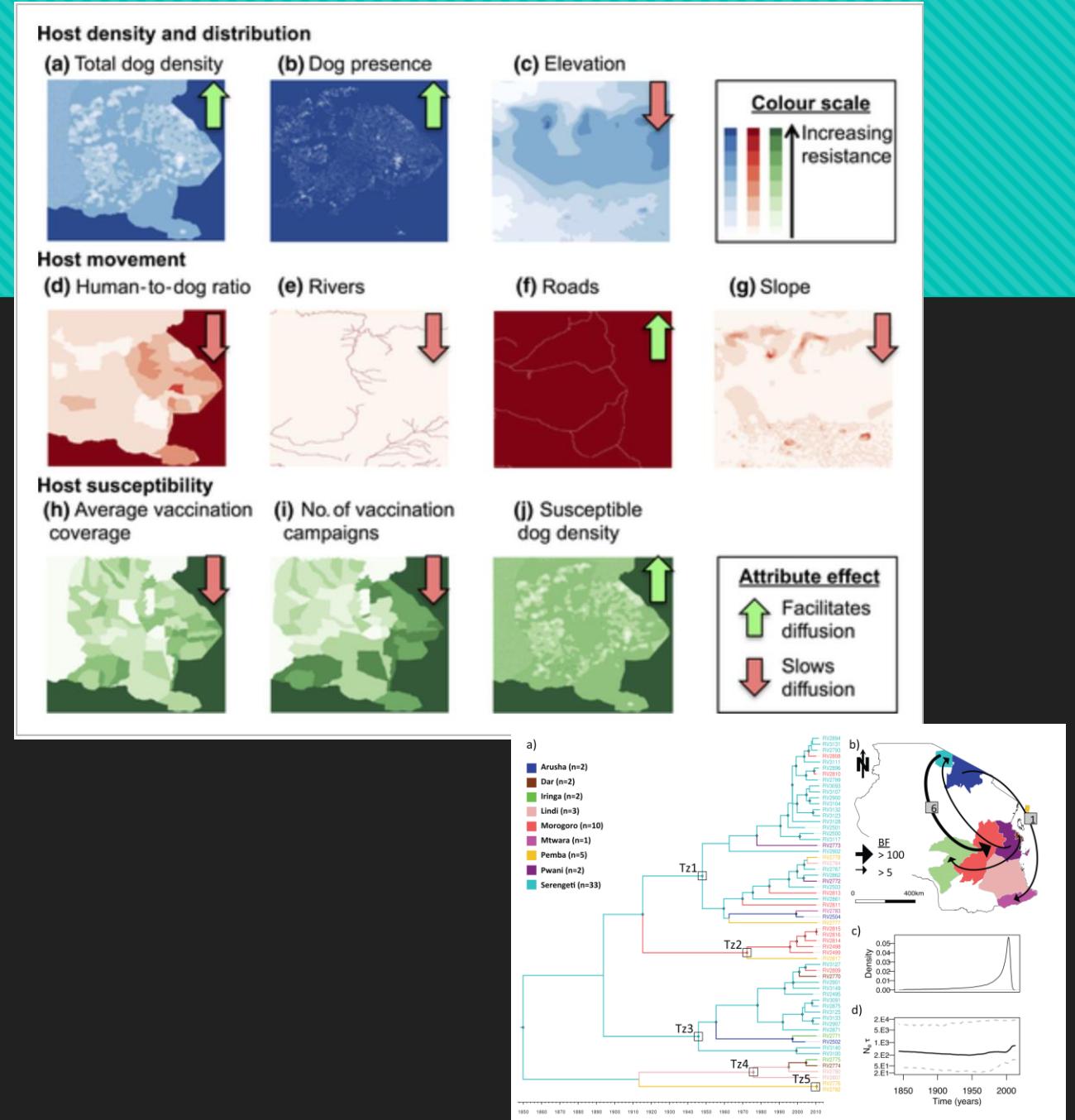


Image copyright [www.cronodon.com](#), used with permission

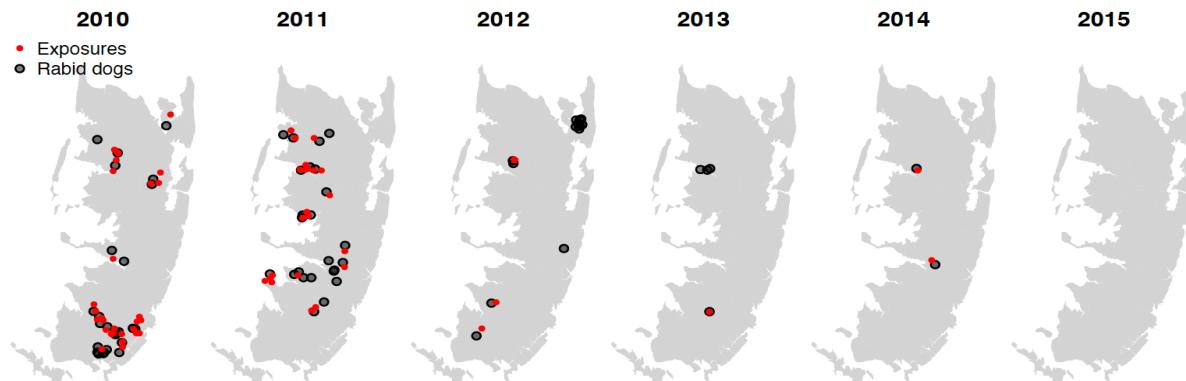
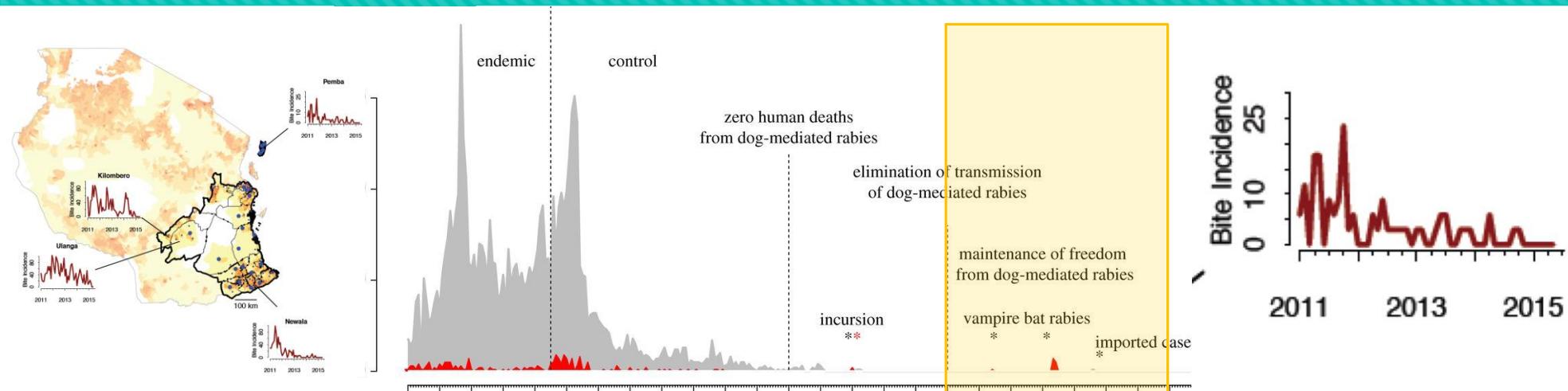
In depth analysis

- Phylodynamic analyses
- Quantifying patterns of spread
- Identify factors driving transmission
 - What drives long-distance movements?

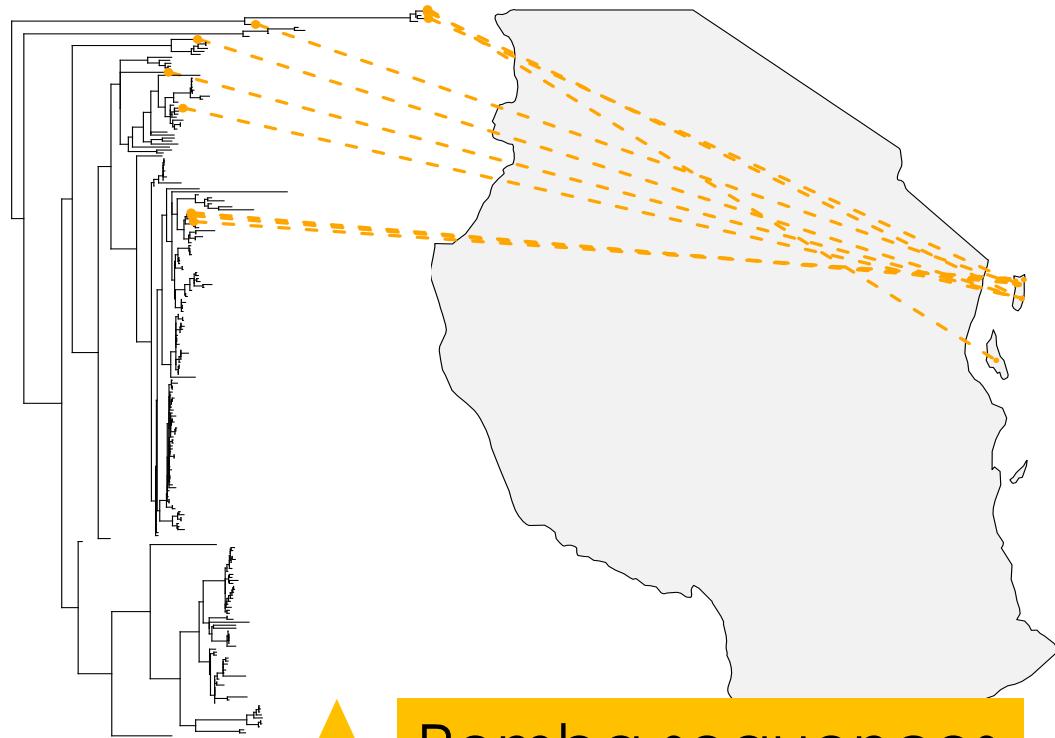
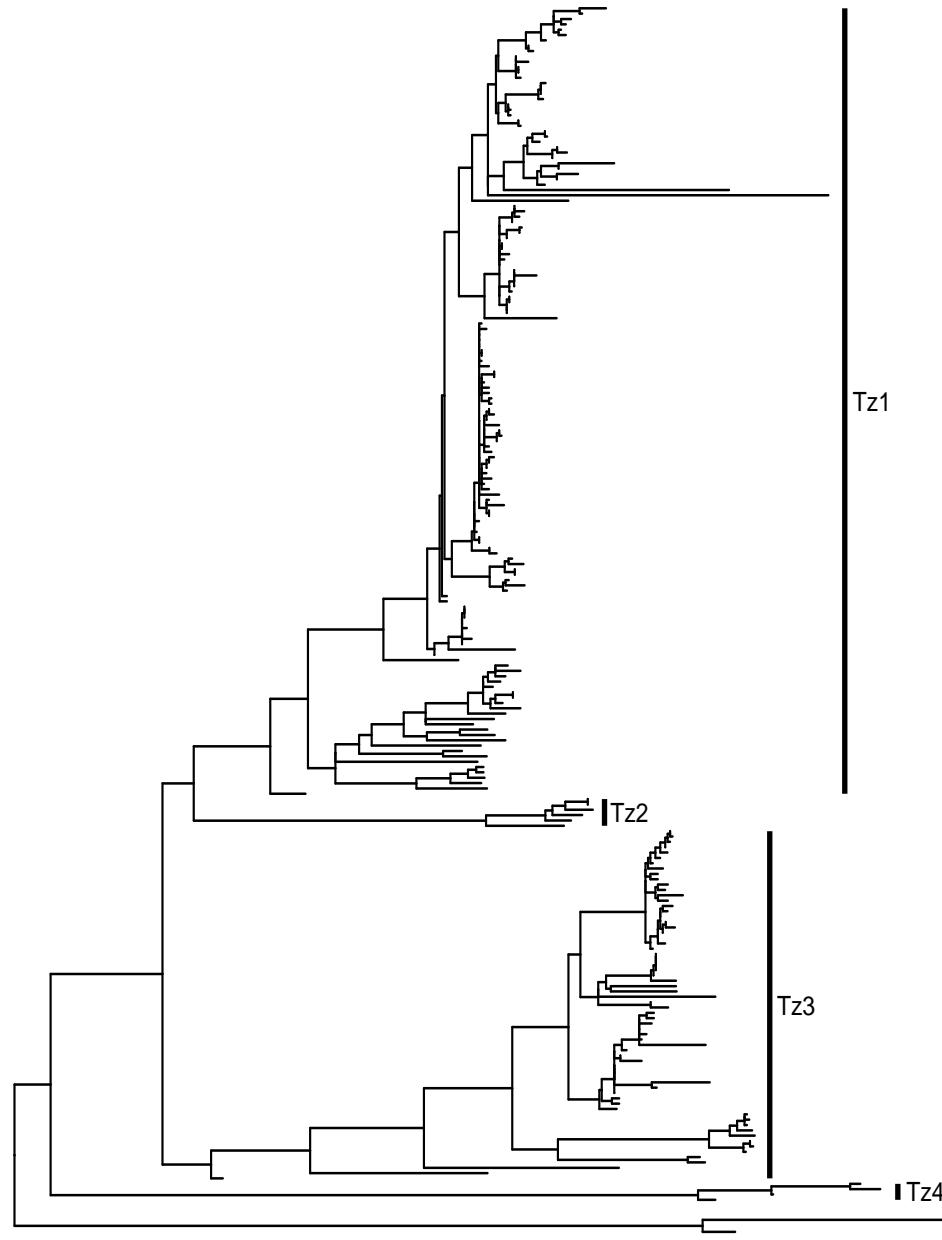


Maintaining freedom

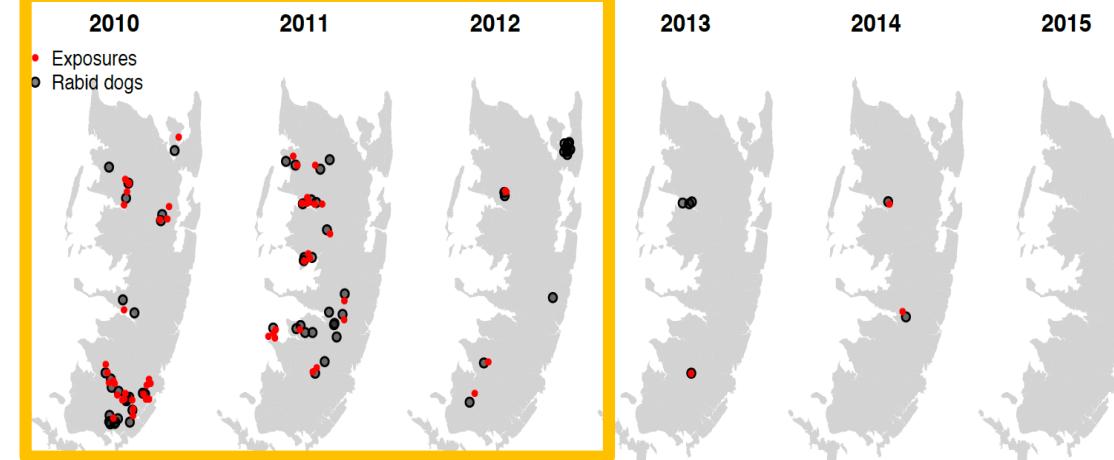
Pemba, Tanzania



Rabies
outbreak
Late 2016-17



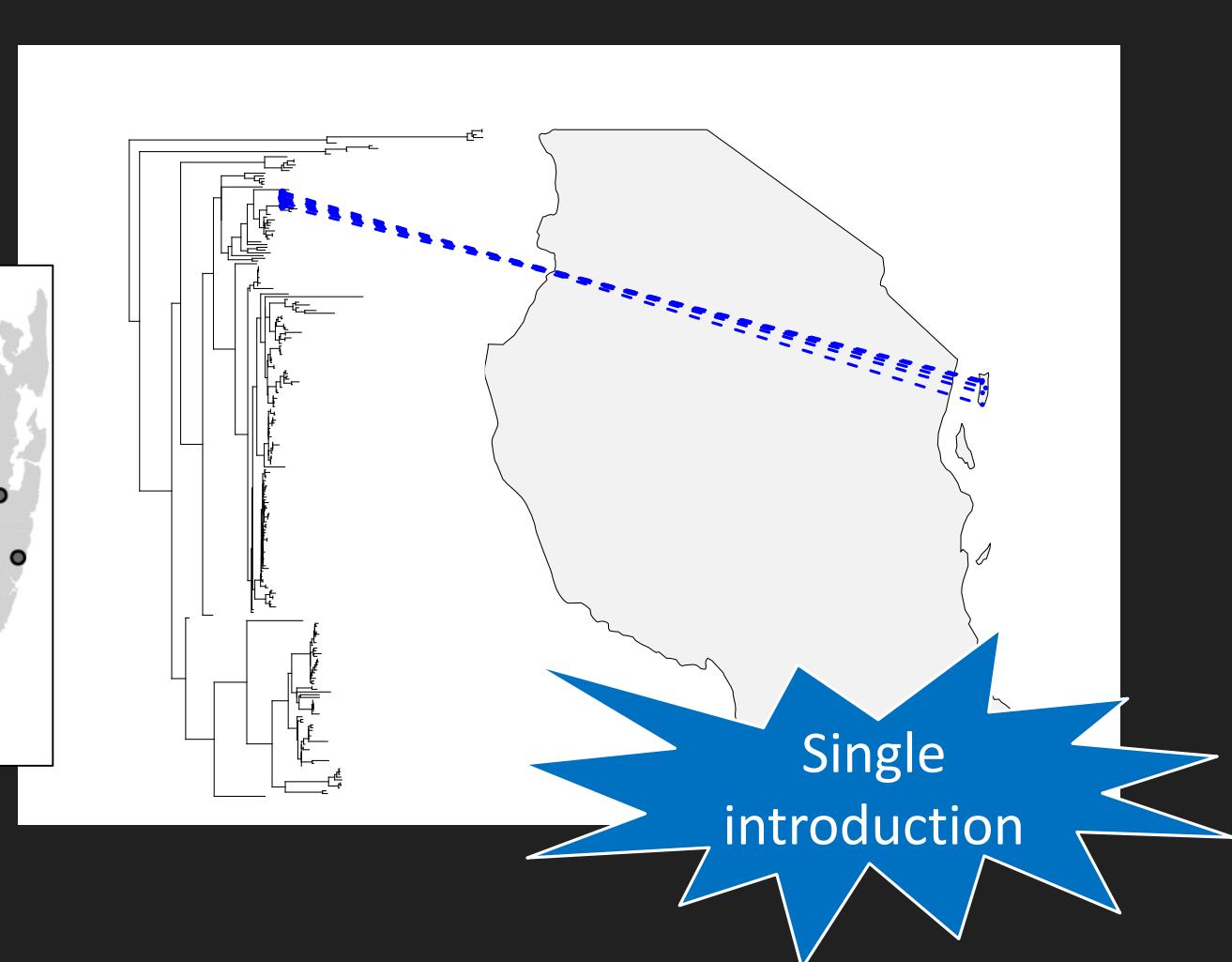
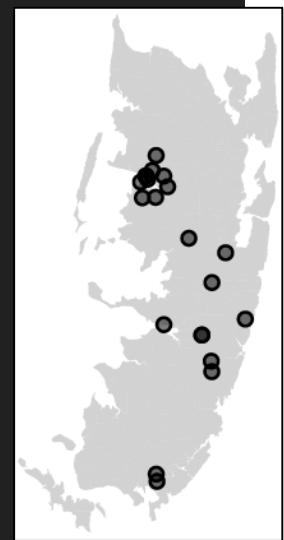
Pemba sequences



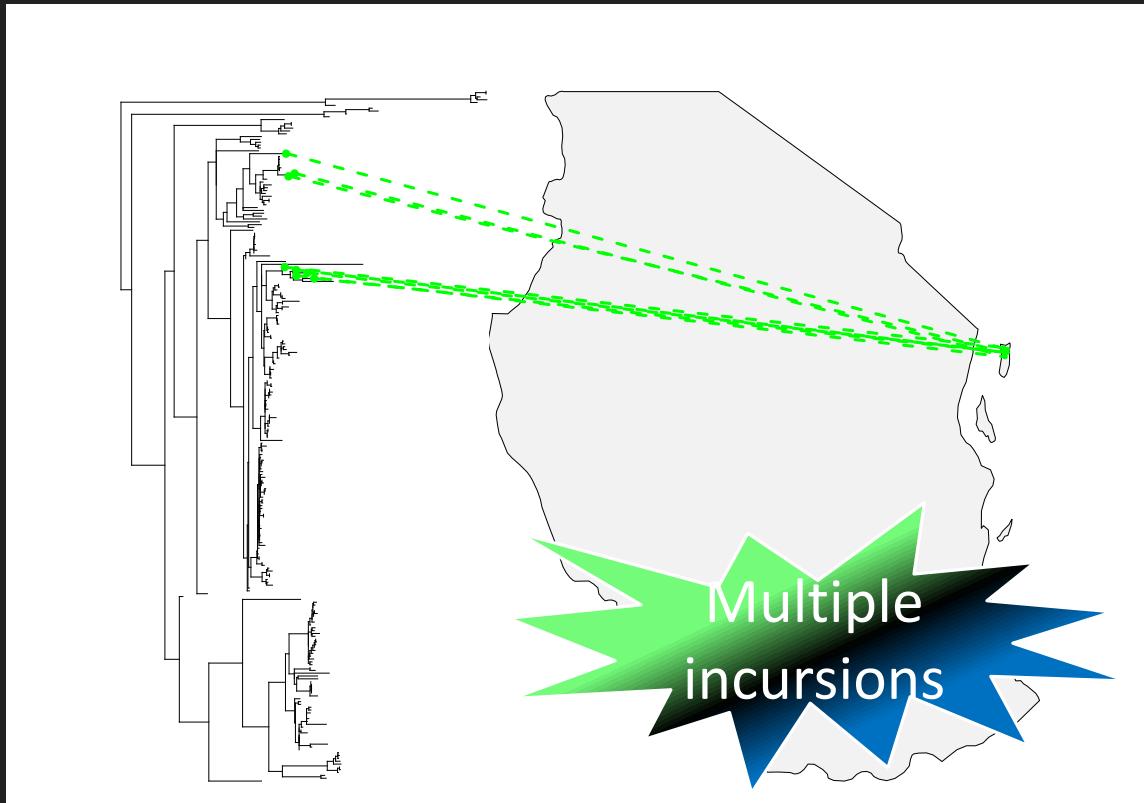
Pemba outbreak 2016: initial surveillance



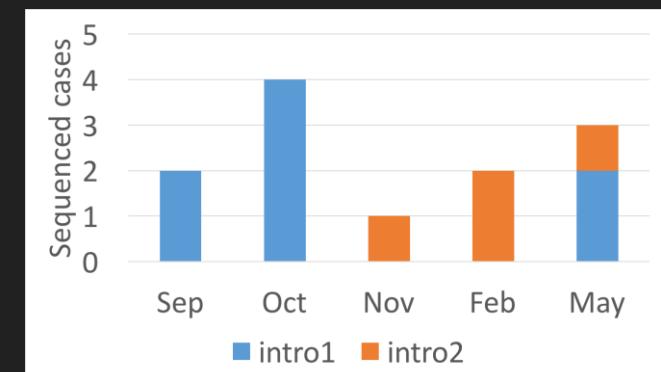
- 6 samples (from Sep/Oct) exported to UK for genetic analysis
- Priority... but took ~4months from case detection



Pemba outbreak 2016: continued surveillance



- 8 new sequences sequenced in the field
- 2 day turnaround

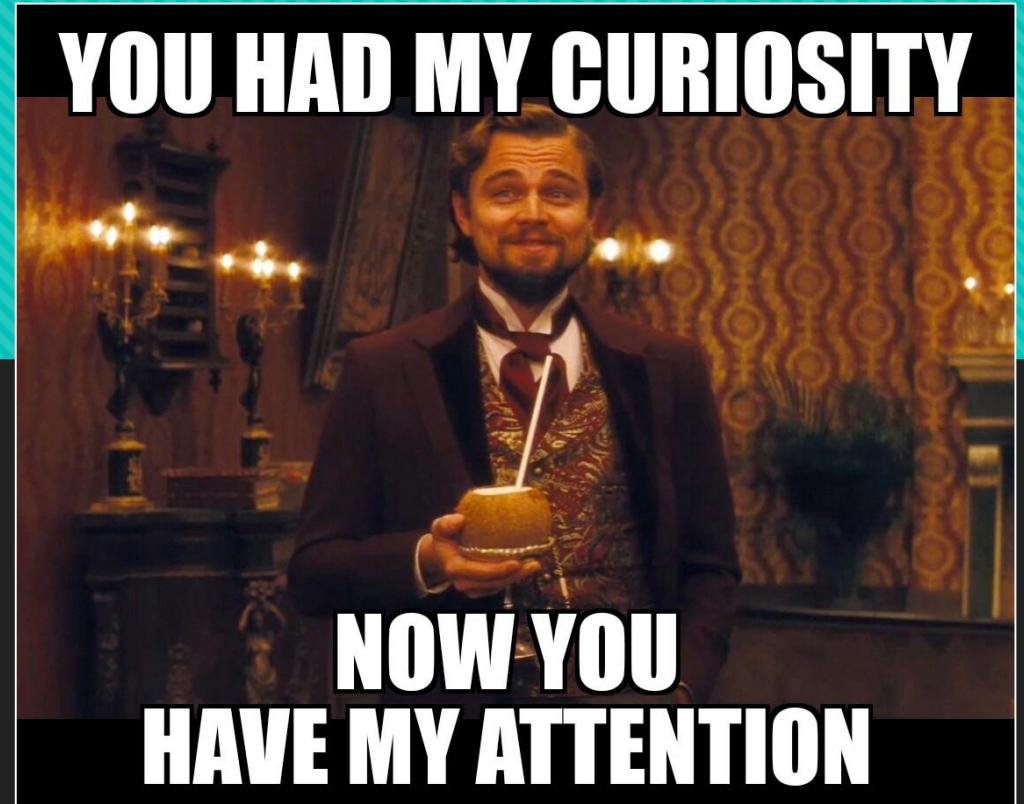


Costs

- Costs vary according to:
 - Protocol used
 - Ability to bulk buy
 - What parts of pipeline are considered

e.g. Sample to sequence on MinION

Multiplex Capacity	Flowcell bulk buy	Consumable	Cost per flowcell (£GBP)	Cost per sample (£GBP)
6-12	12	Flowcell	617	78-133
96	12	Flowcell	617	30
12	48	Flongle	100	30
96	48	Flongle	100	23



Illumina NextSeq

£50-70 per sample
(Considering 384 samples on one run.
Includes DNA library prep to sequence)

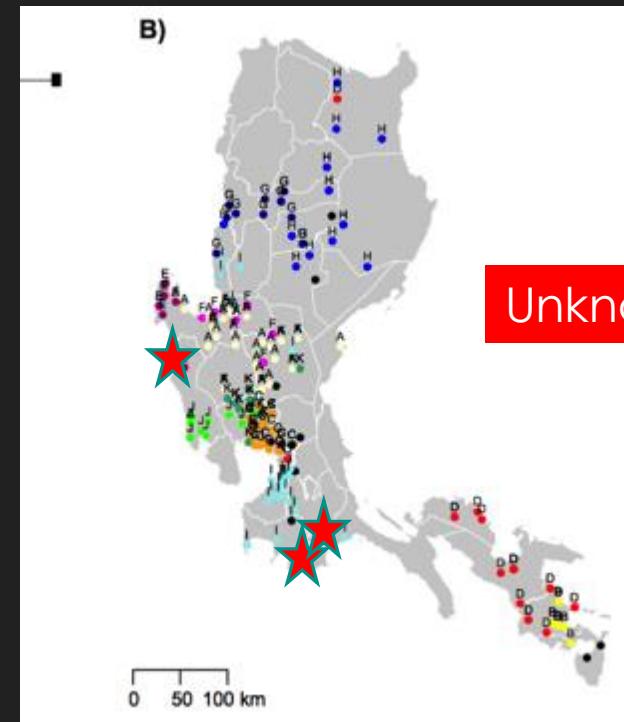
Best case scenario

Training

- Training workshop in 2019
- Hands on experience with MinION sequence
 - Sample to sequence prep
 - Basic analysis



Wee demo...



Whole genomes in Asia

- 84 WGS (with at least 90% genome coverage)
- 9 countries
- Distinct Philippines subclade