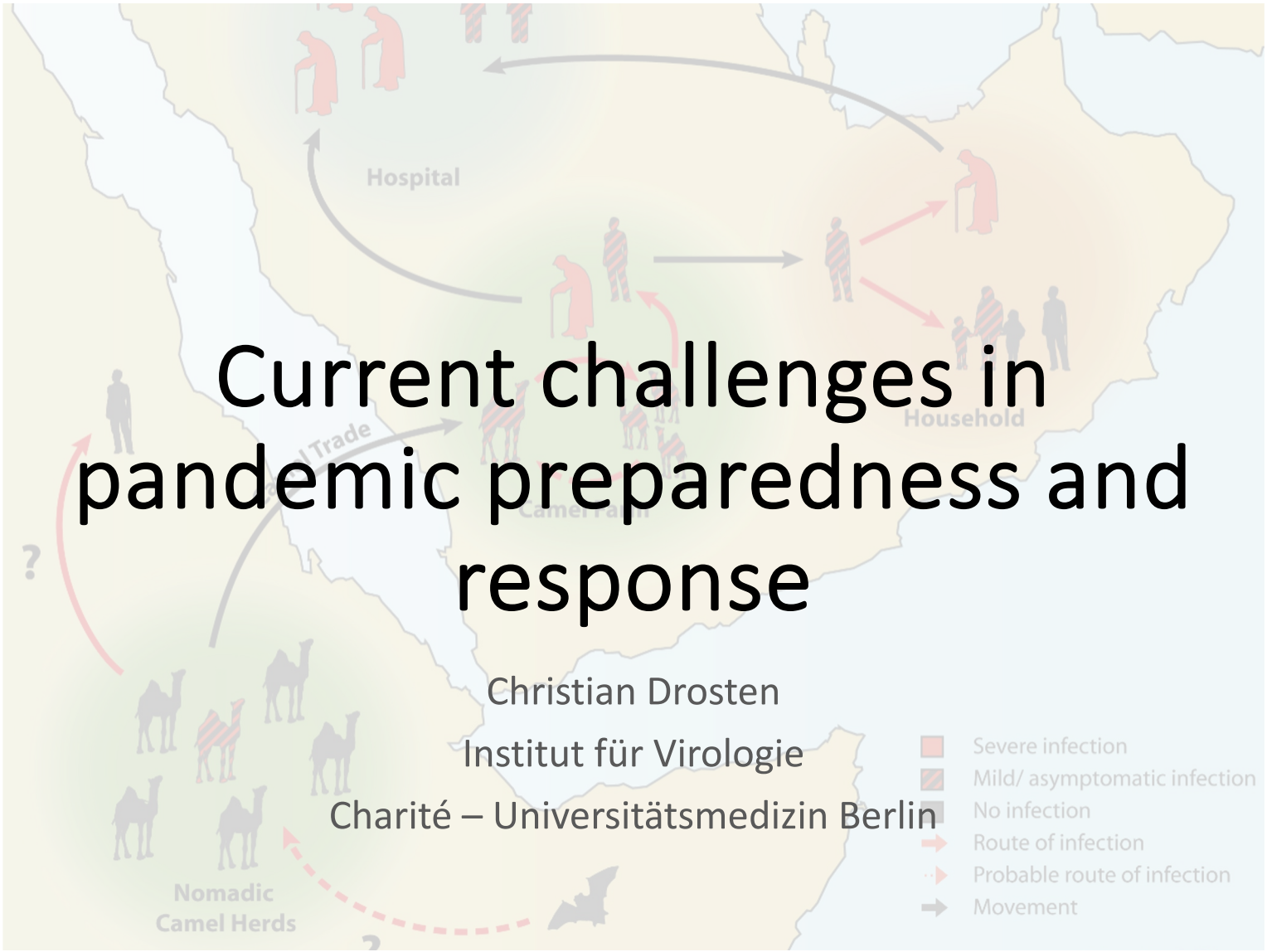
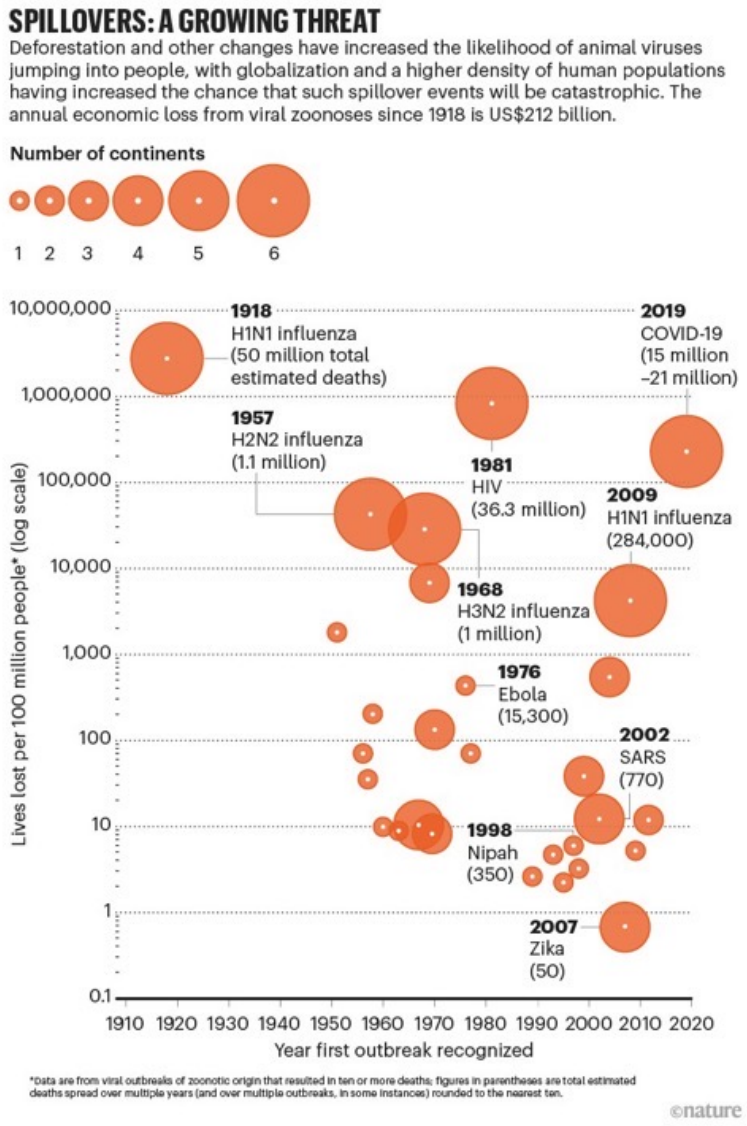


Current challenges in pandemic preparedness and response

Christian Drosten

Institut für Virologie

Charité – Universitätsmedizin Berlin



Vora, Nature 2022

1918: „Spanish Flu“, Influenza A H1N1
15 – 50 mio. fatalities



Influenza A: under surveillance

Avian high- and low pathogenic influenza A with human cases or limited outbreaks

- H5N1
- H7N9
- H5N6
- H7N7
- H7N3
- H7N1
- H5N8
- H9N2

Relevant types in swine:

- H1N2v, H3N2v, etc.

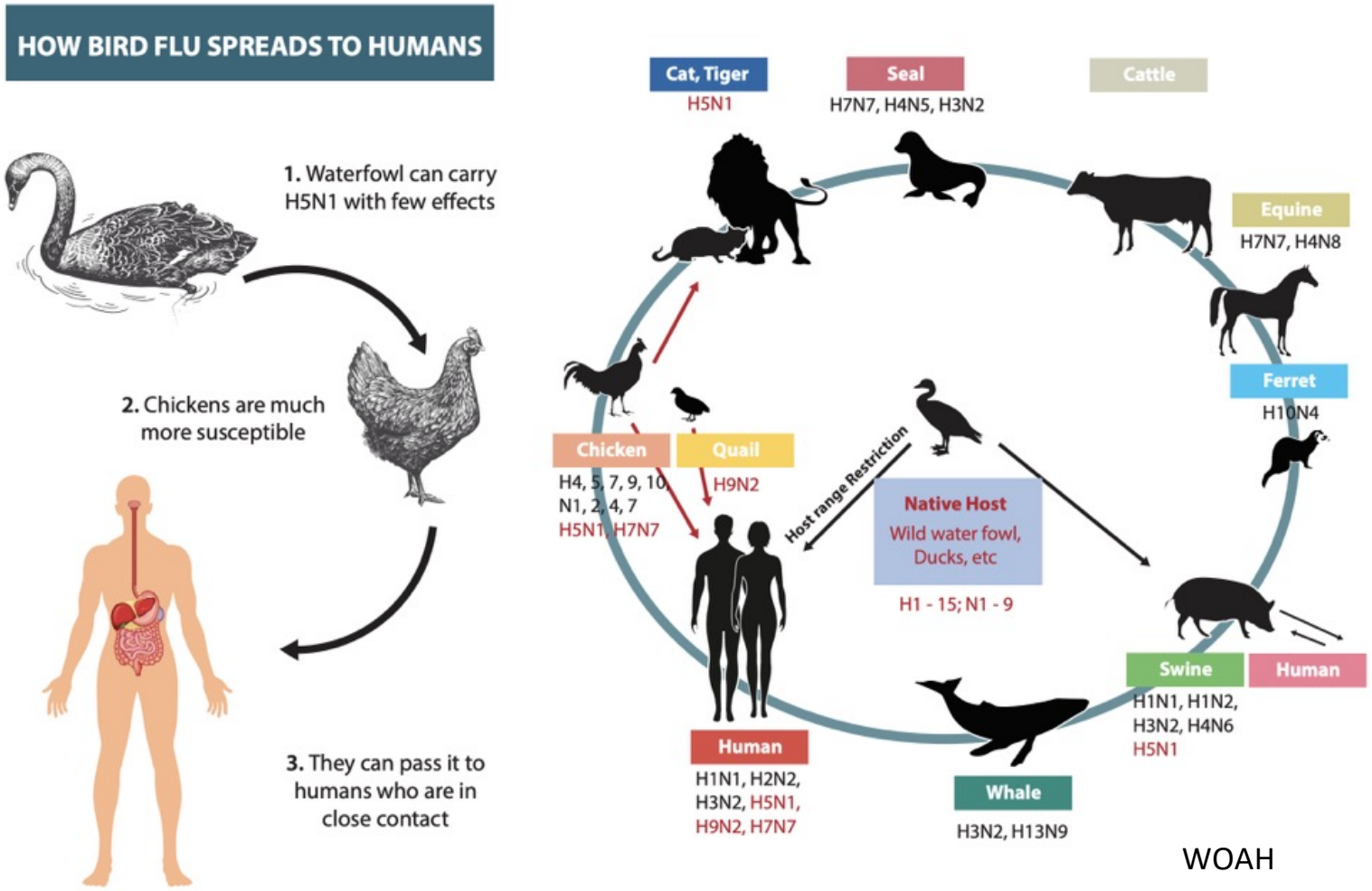
Sources of influenza A viruses – classical concept







Direct mammalian acquisition of avian influenza A viruses

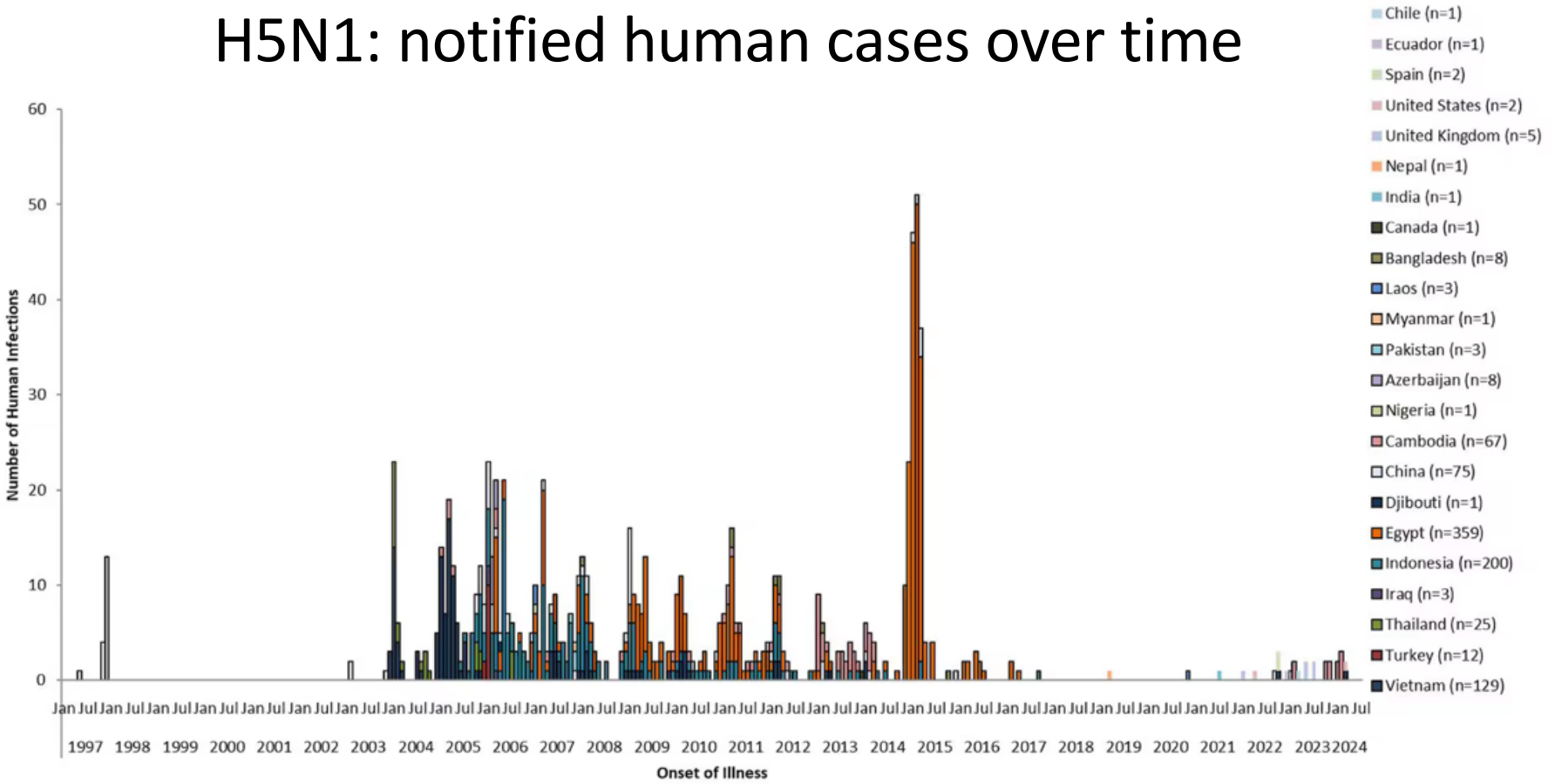


WOAH

H5N1 sporadic human cases, 2003 - 2024

- Cases: 876
- Fatalities: 458
- Case fatality rate: 52%
- Strong underascertainment likely: exposure-based surveillance
- No signs of human-to-human transmission

H5N1: notified human cases over time


Number of Human Infections
0
10
20
30
40
50
60
Jan Jul Jan Jul Jan Jul



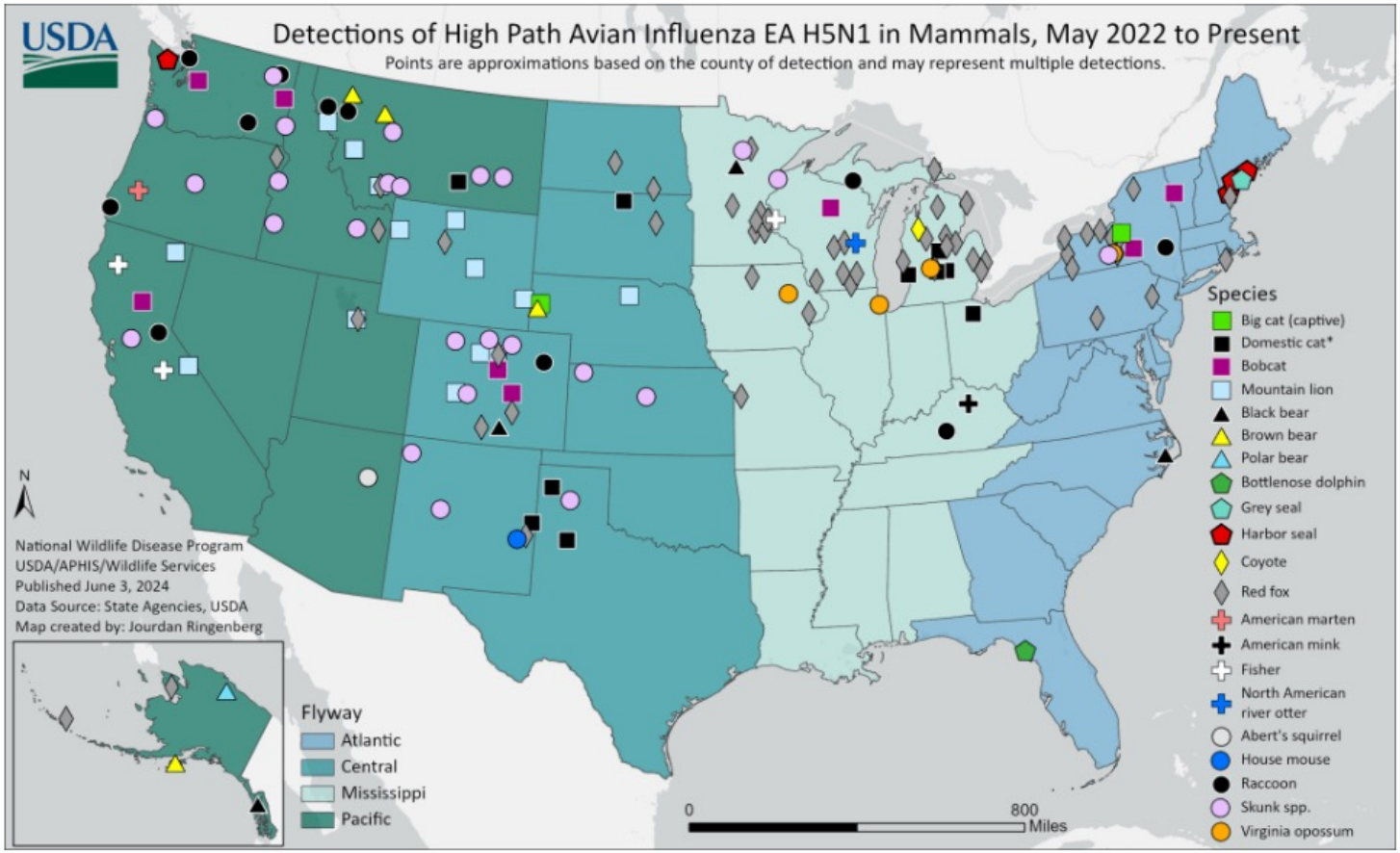
Commercial fur farms

H5N1, Clade 2.3.4.4b: increasing frequency of outbreaks in mammals



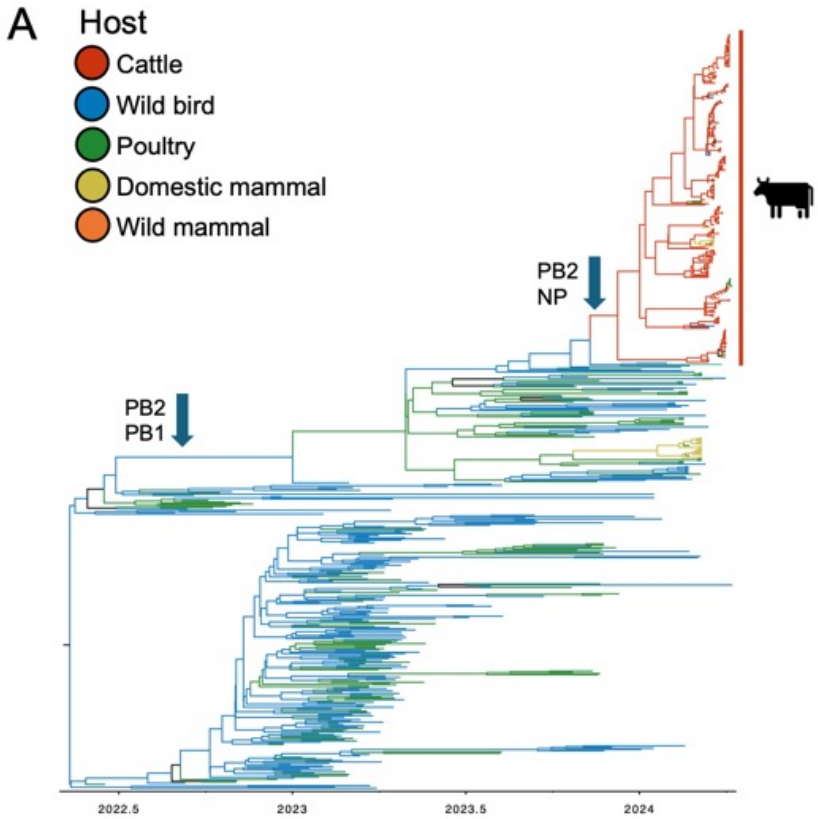
Feral carnivores

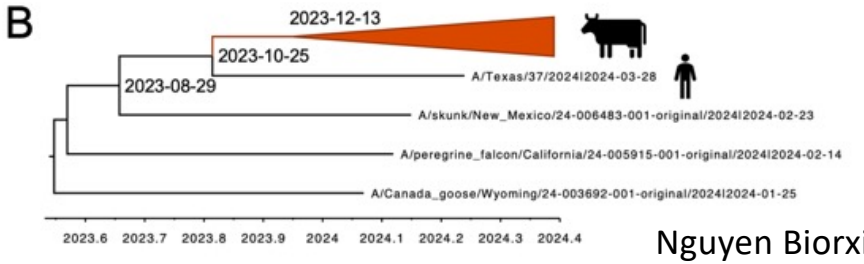
Sources: AAAS; New Scientist



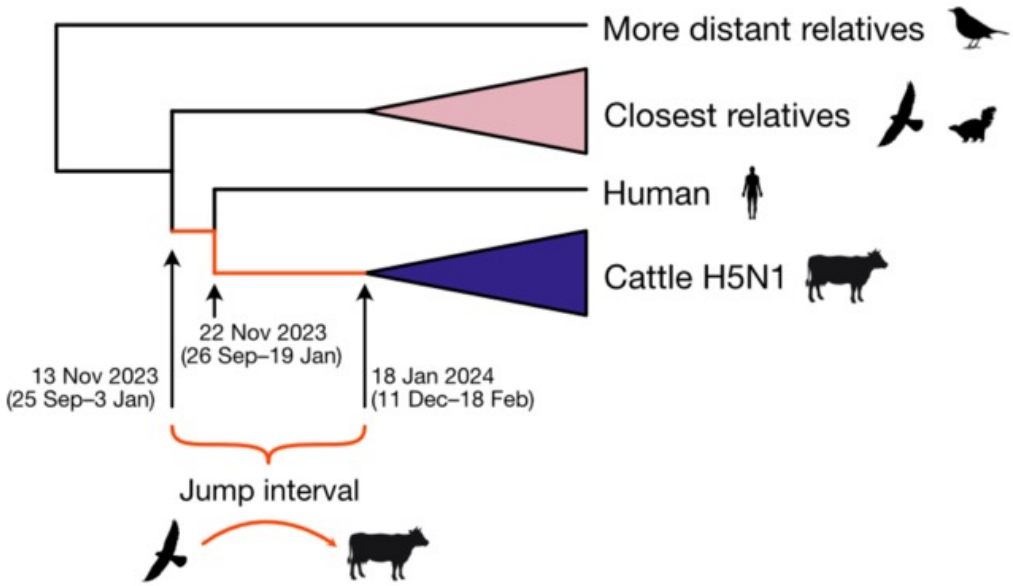
Update June 3, 2024

Single introduction in cattle, ca. October-January 23/24

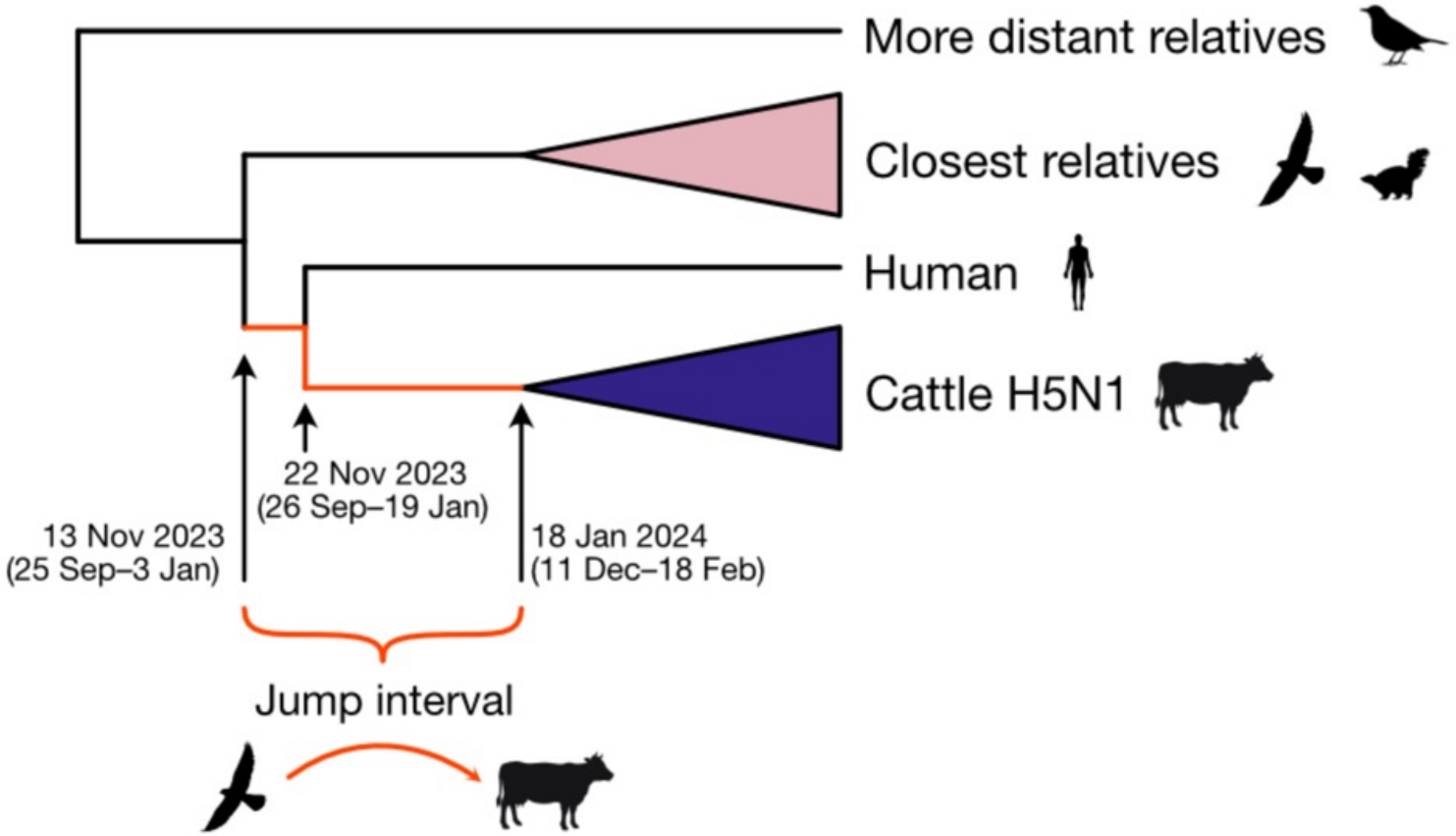




Nguyen Biorxiv 2024.05.01.591751v1



Worobey, Virological.org May 3rd, 2024



Worobey, Virological.org May 3rd, 2024

Bird- and mammal flu receptor side by side in bovine udder tissue

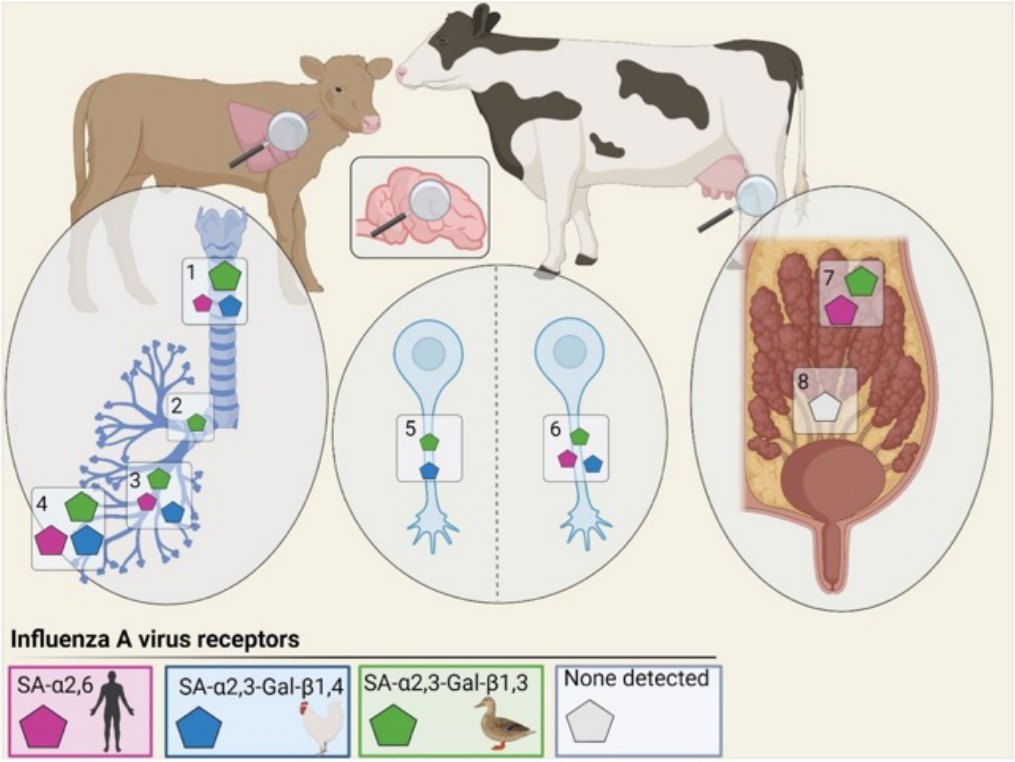
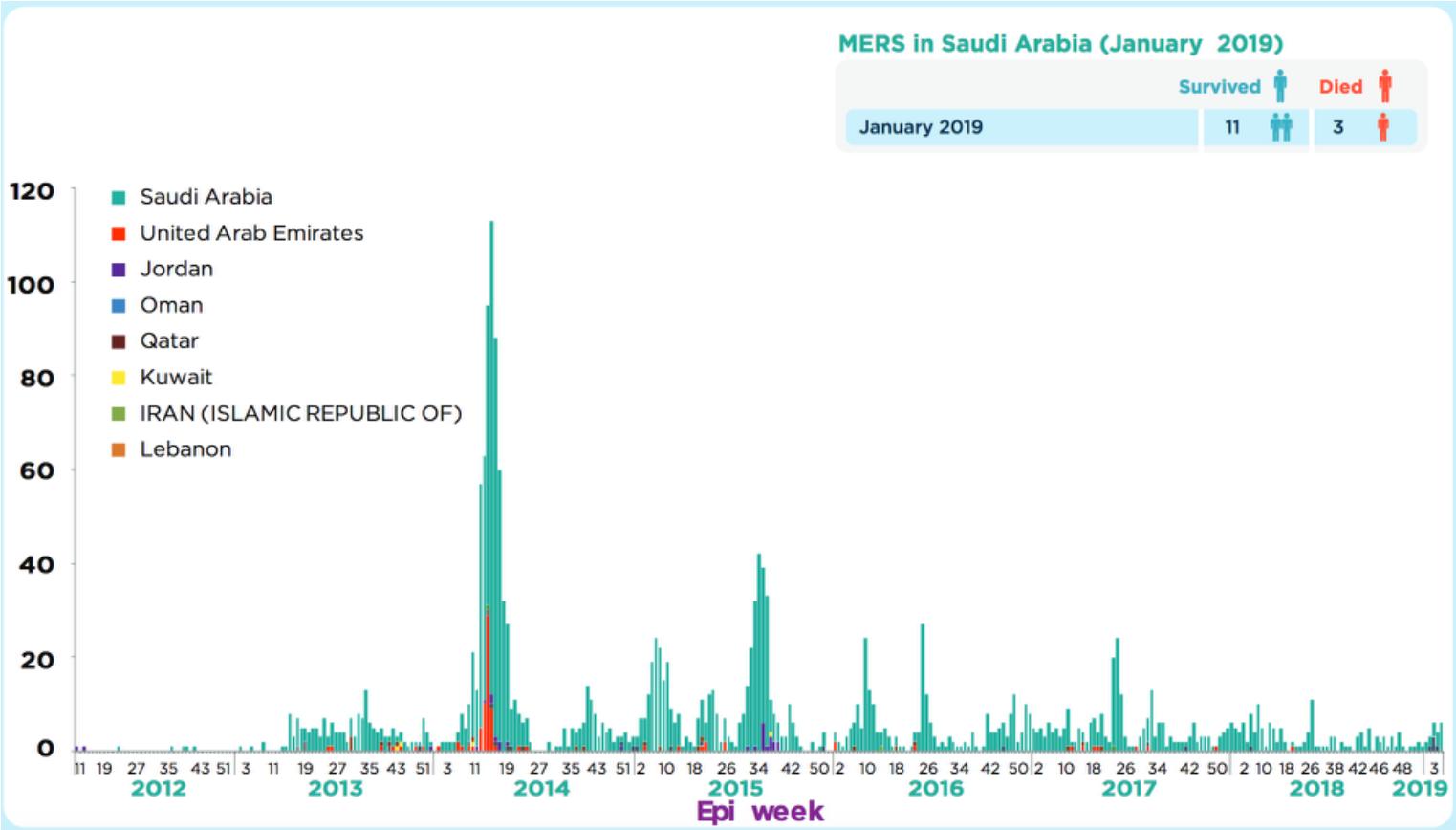


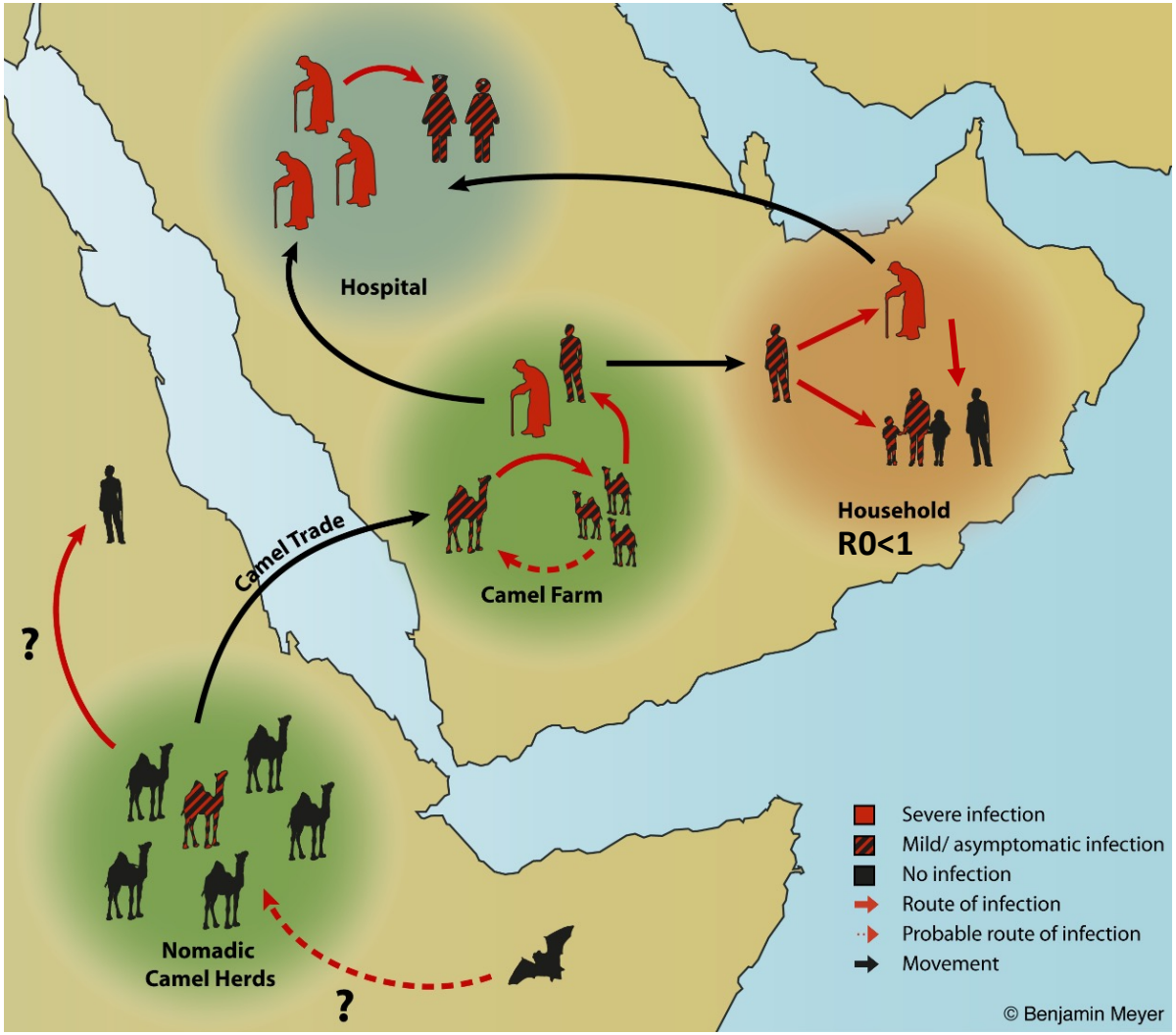
Figure 3. Comparison of the distribution of influenza A virus (IAV) receptors in the lungs, cerebrum, and mammary gland tissues of cattle. The distribution and staining intensity of the IAV receptors was detected by lectin histochemistry and semi-quantified. The human receptor (pink) was detected by Sambucus Nigra Lectin (SNA), the chicken receptor (blue) was detected by Maackia Amurensis Lectin I (MAA-I), and the duck receptor (green) was detected by Maackia Amurensis Lectin II (MAA-II). The tissues investigated were the trachea (1), bronchi (2), bronchioles (3) alveoli (4) of the bovine respiratory tract, and the cerebellar cortex of a beef calf (5) and a dairy cow (6) and lastly the alveoli (7) and ducts (8) of the mammary gland. Created with Biorender.com.

Kristensen, BioRxiv 2024.05.03.592326

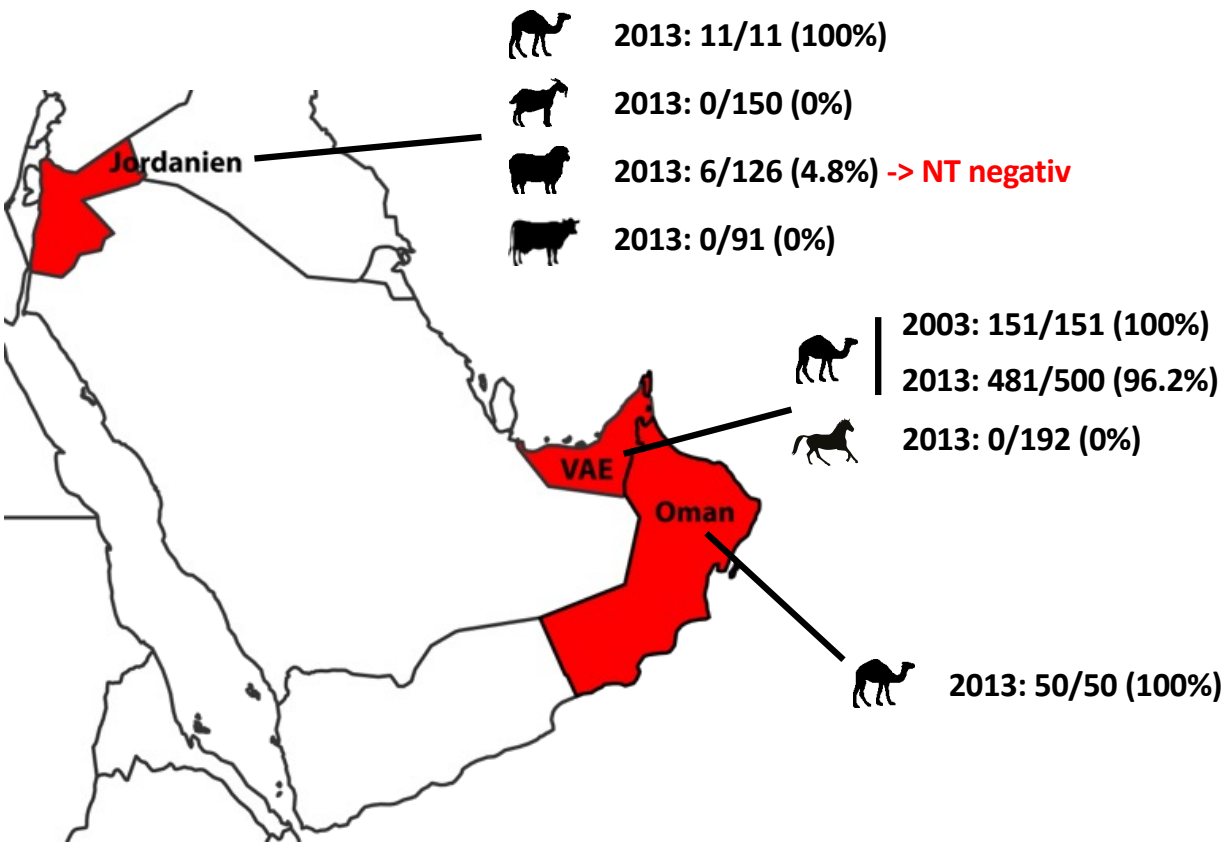
MERS: >2298 cases, >811 fatalities



Source: WHO EMRO

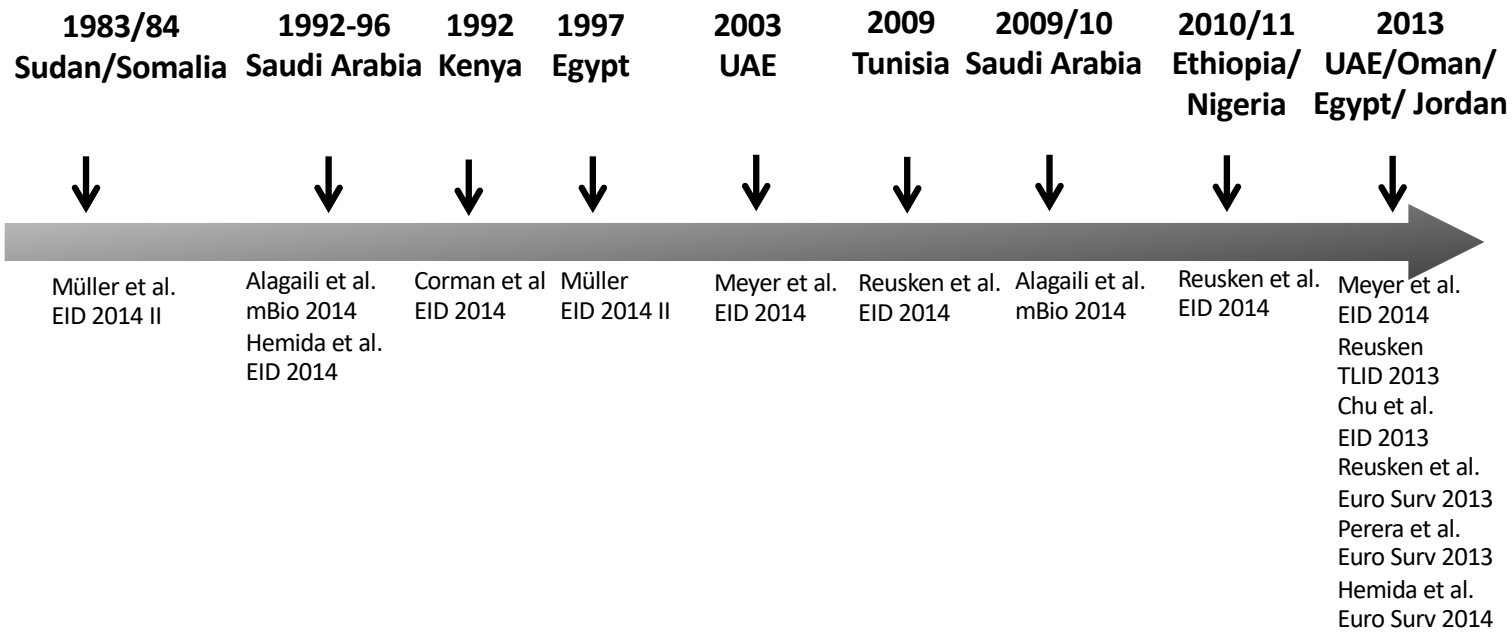

Hospital
Household
R0<1
Camel Farm
Camel Trade
?
Nomadic
Camel Herds
?
Severe infection
Mild/ asymptomatic infection
No infection
Route of infection
Probable route of infection
Movement
© Benjamin Meyer

Dromedaries have MERS-specific antibodies at high prevalence rates

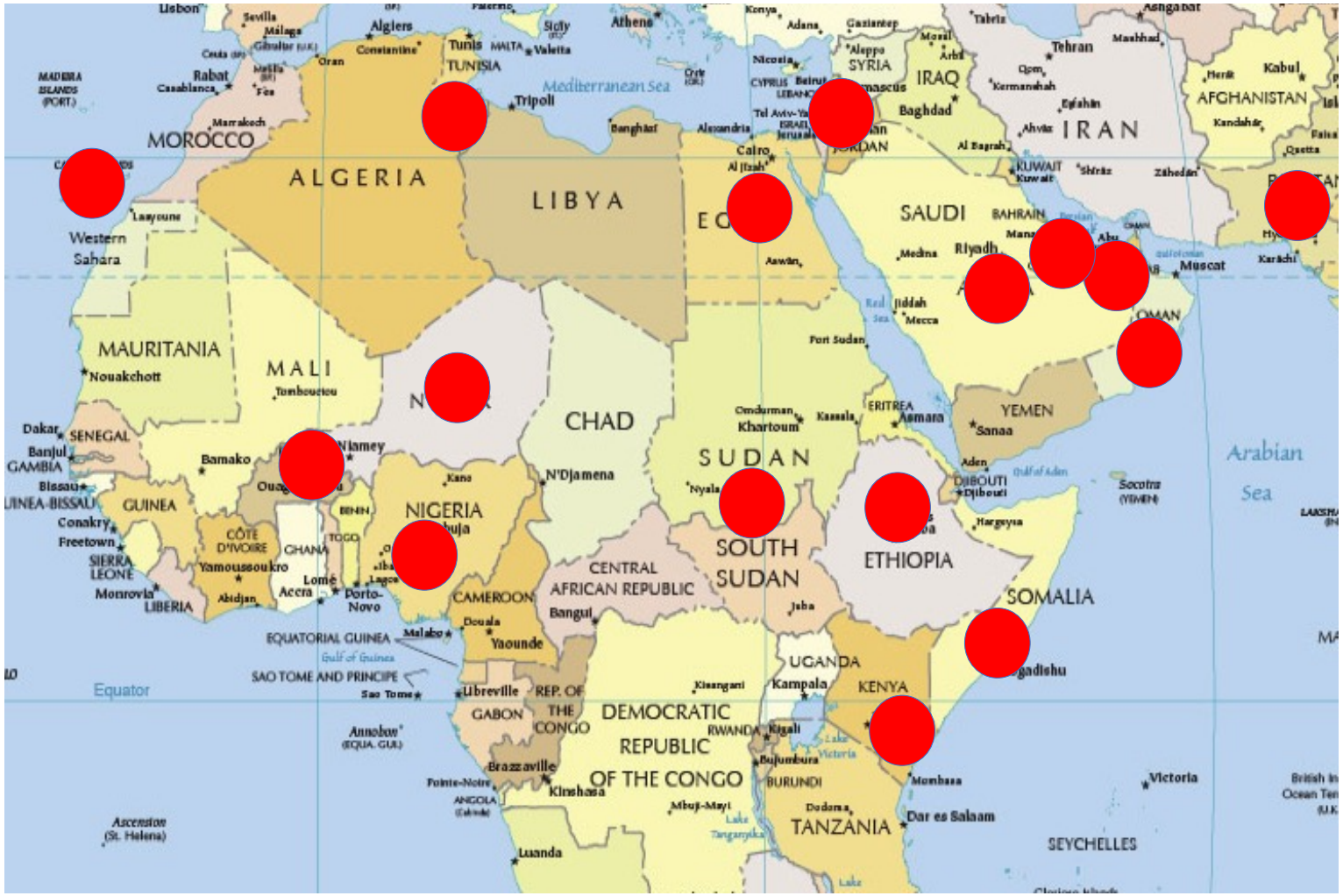


Meyer et al., Emerging Infectious Diseases 2013; Meyer et al., Emerging Infectious Diseases 2014; ; Reusken et al., Euro Surveillance 2013; Reusken et al., The Lancet Infectious Diseases 2013

Seropositivity in dromedaries since >30 years



Proof of MERS-CoV infection enzootic in dromedary camels





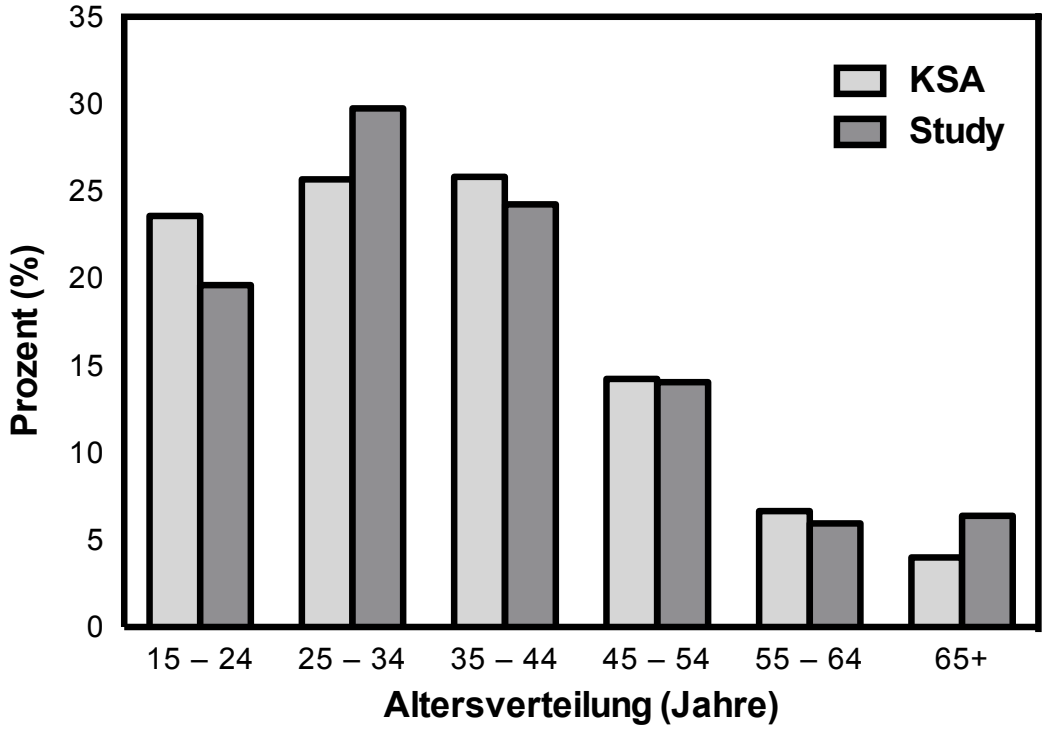
Serosurvey in Saudi Arabia





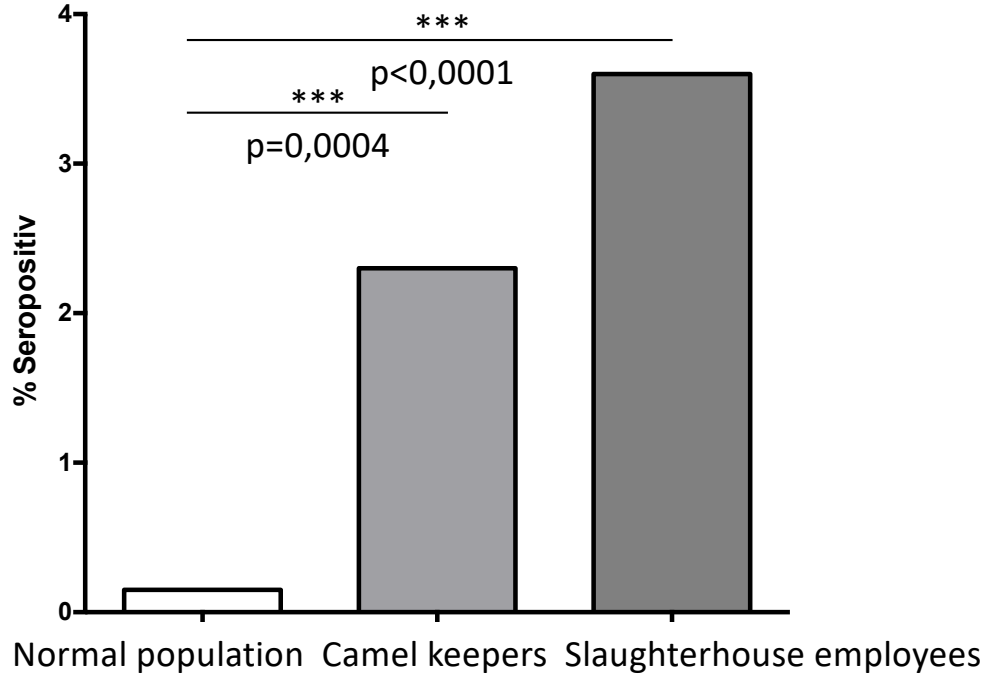
Photo: Marcel Müller, Virology Bonn

Age distribution, study vs. KSA population



Müller, Meyer et al., The Lancet Infectious Diseases 2015

Human seroprevalence in three cohorts

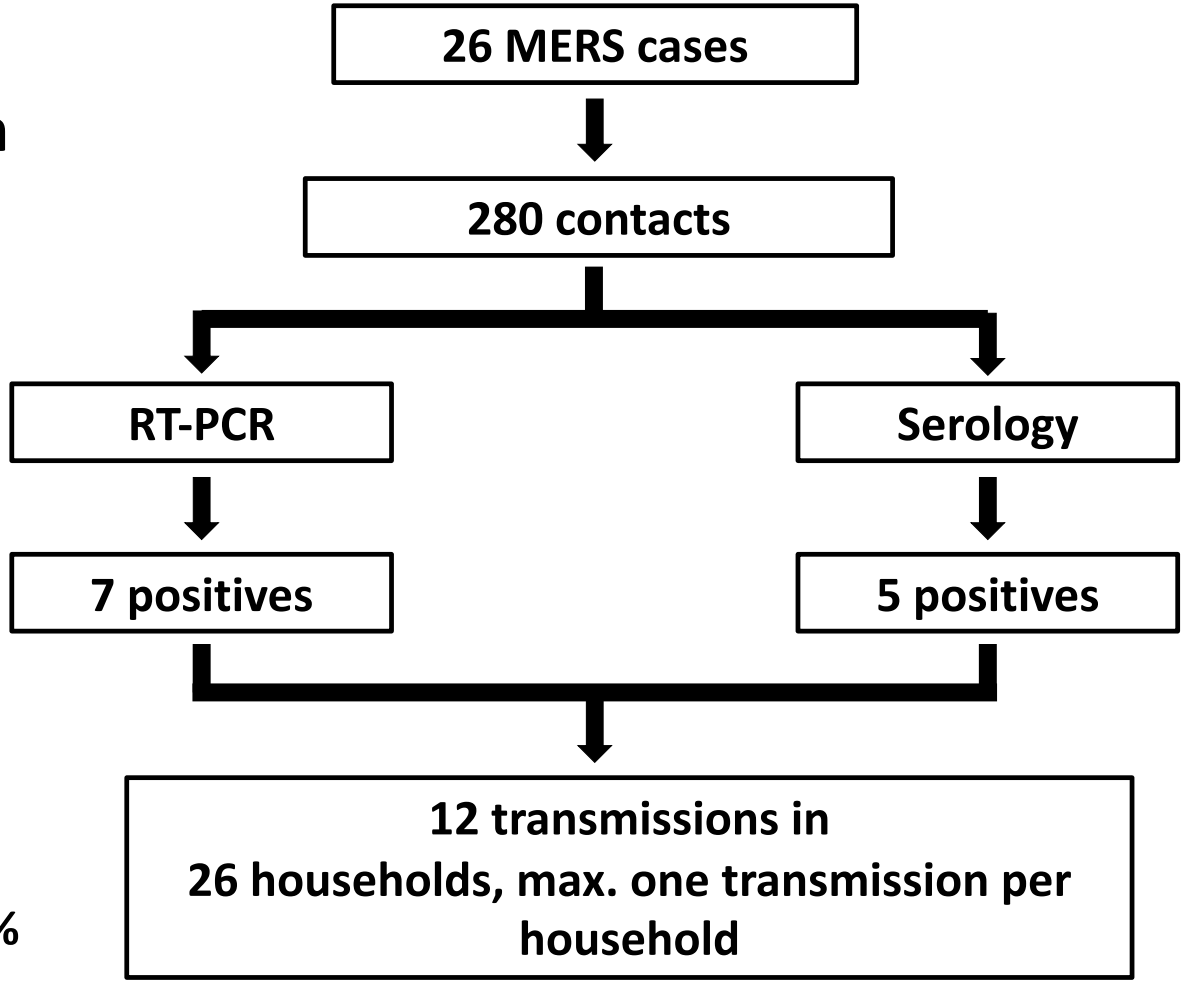


0.15% seroprevalence in normal population

Implicates ca. 40,000 cases within ca. 5 years in KSA

Müller, Meyer et al., Lancet Inf Dis 2015

Inefficient transmission in households



SAR = 4.3%
(R_0 < 0.5)

Drosten, NEJM 2014

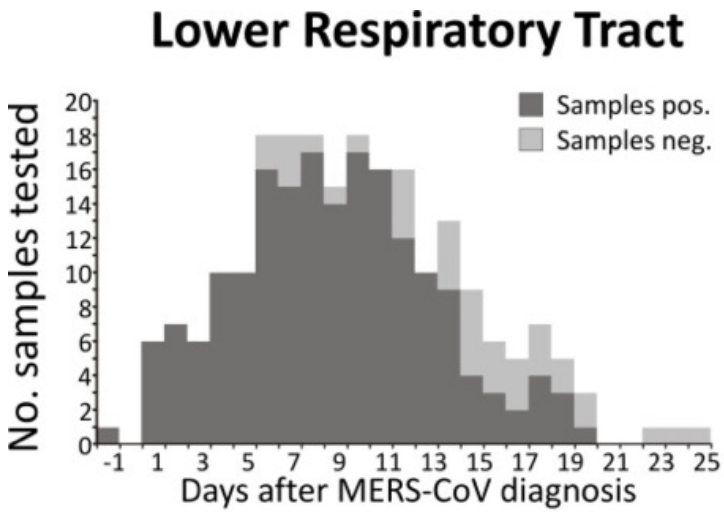


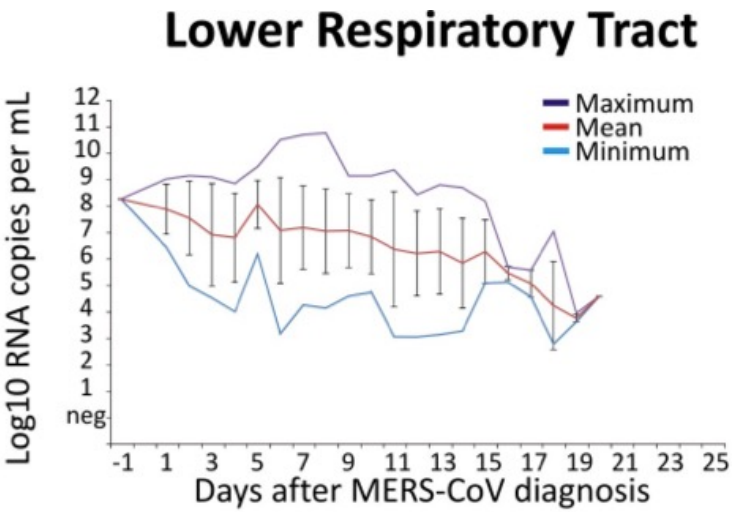
King Fahd Hospital;
Jeddah

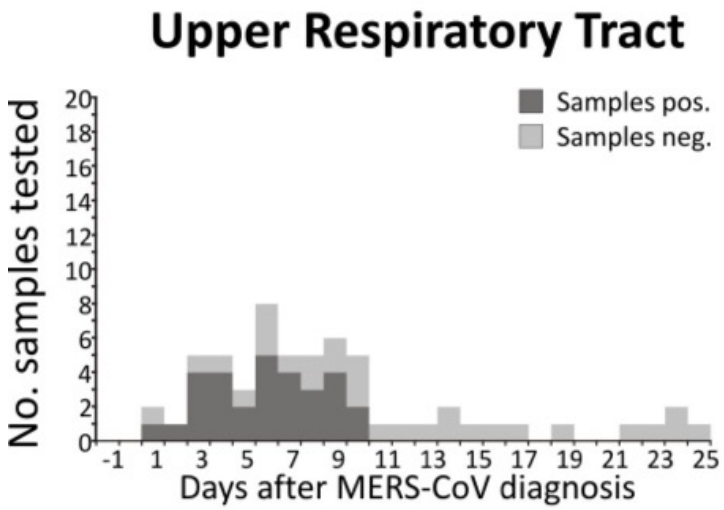
Emergency Department

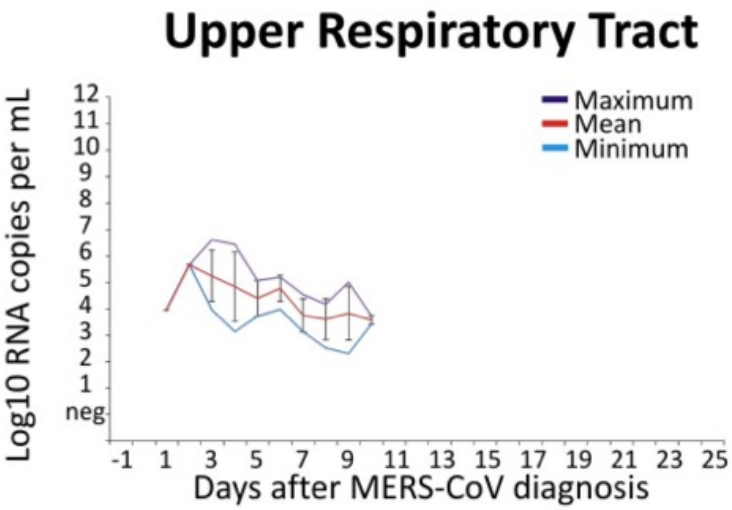




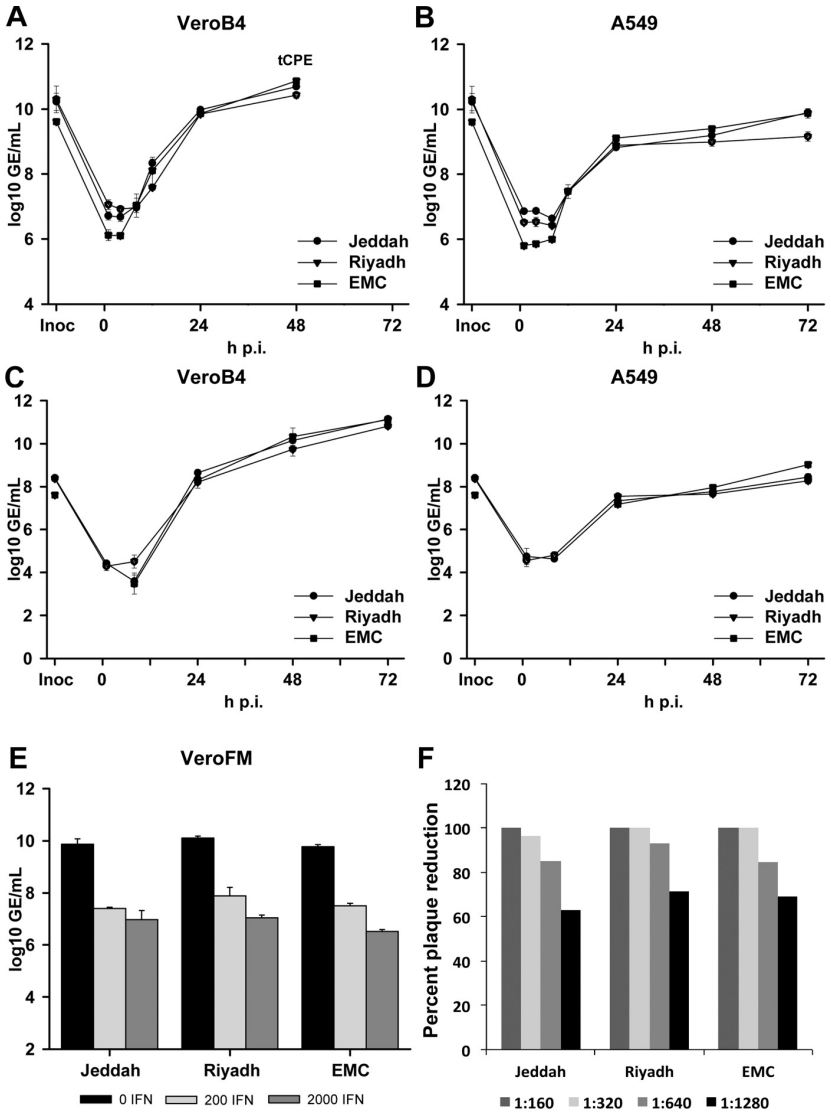








Corman, CID 2015



No change in

-Replication level
-Interferon sensitivity
-Serum neutralization

Drosten, Muth, Corman, Hussain et al., CID 2014

No signs of virus changes in Jeddah 2014 outbreak

Korea 2015 outbreak



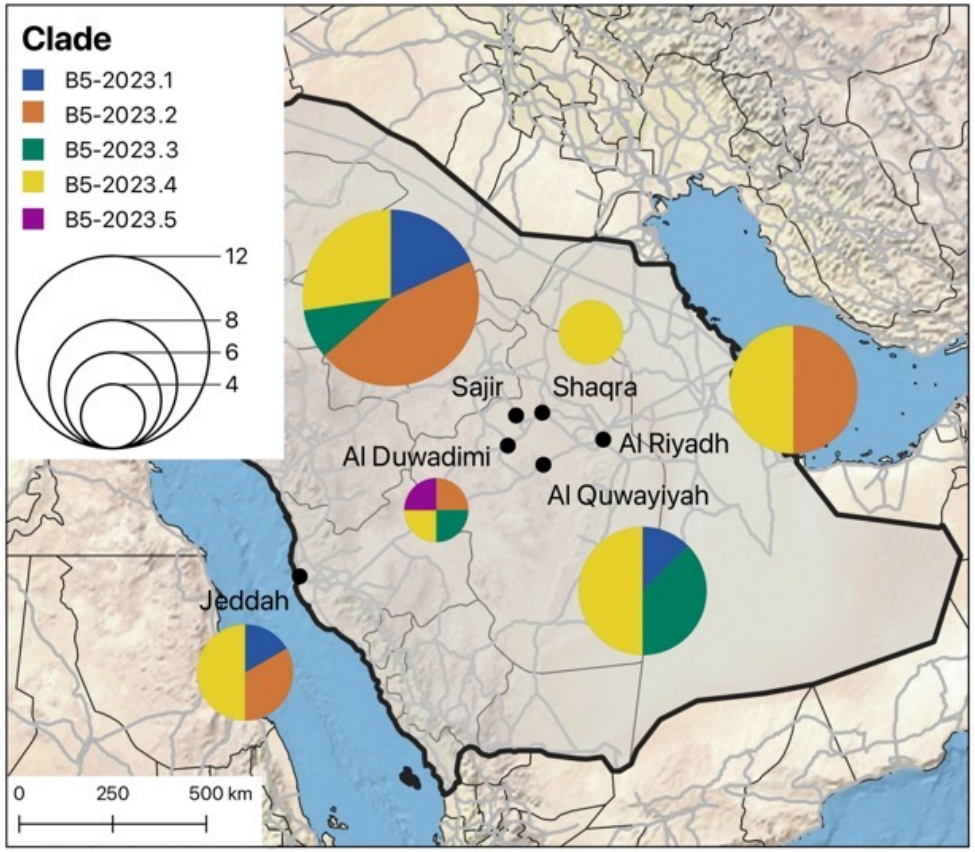


Photo: The Malaysianinsider



Wall Street Journal June 5th, 2015

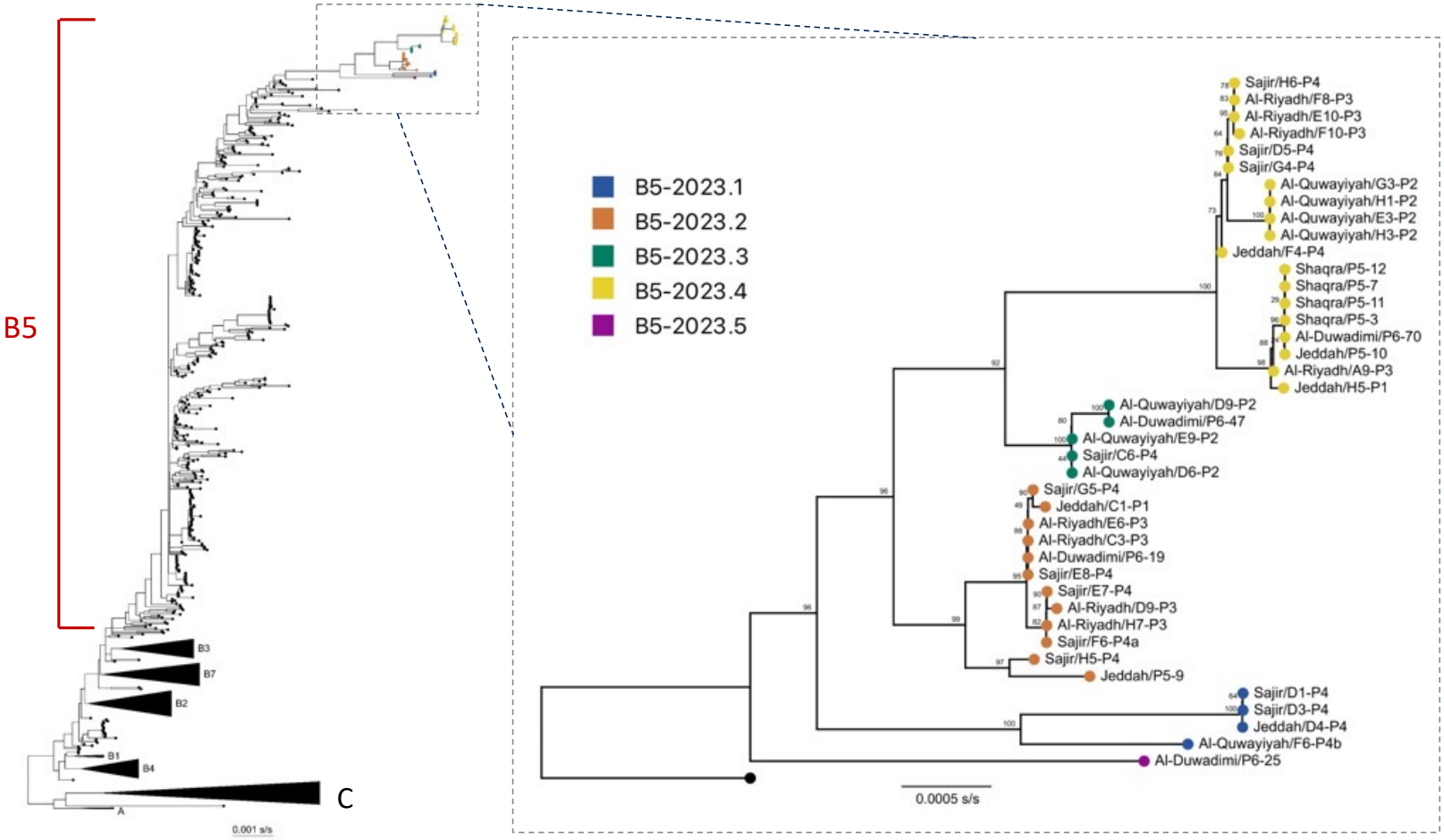
MERS lineages sampled 2023-2024
-onward divergence of lineage B5 into sublineages-



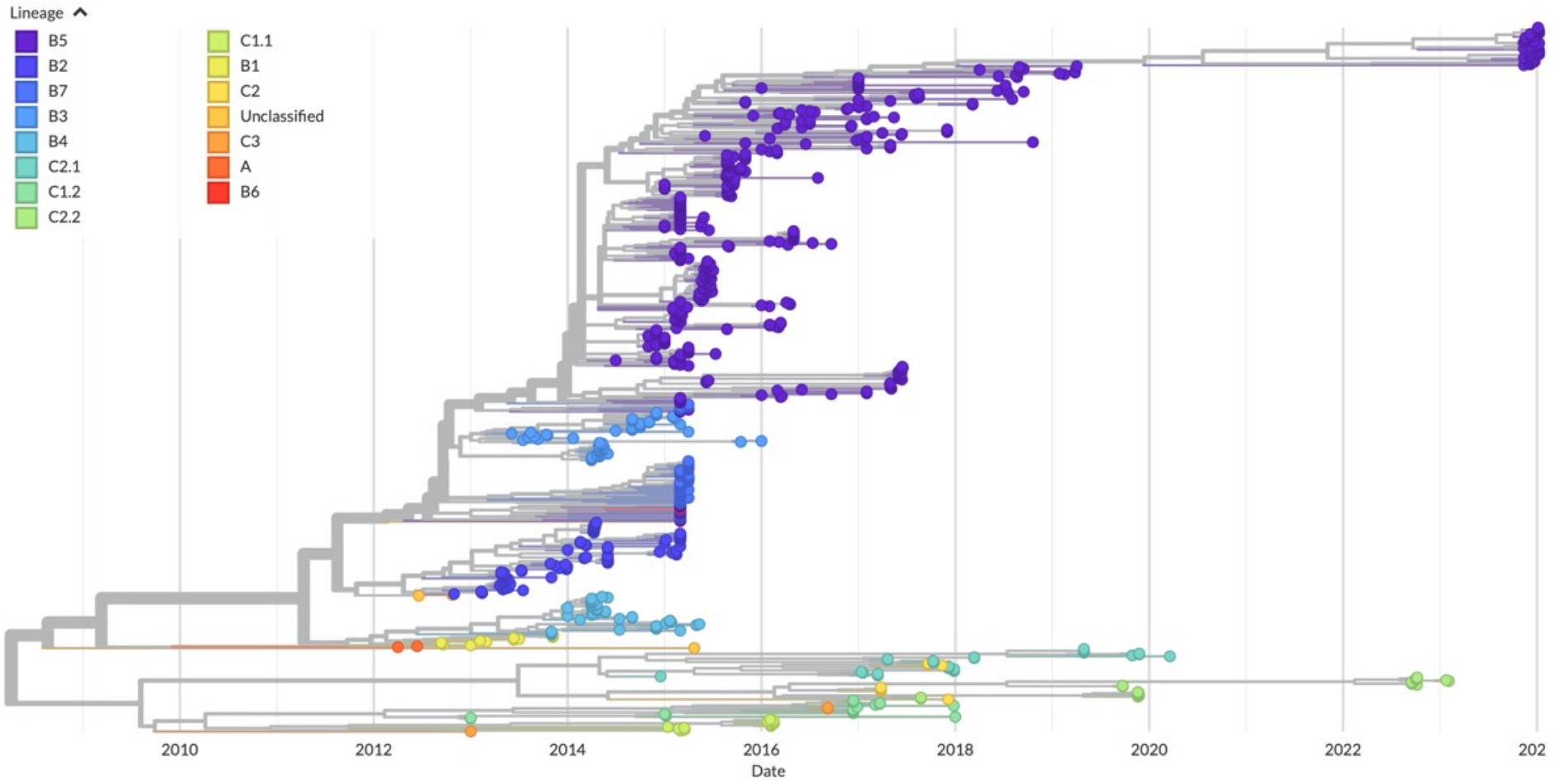
	Total	Riyadh	Jeddah	Quwayiyah	Shaqra	Duwadimi	Sajir
Individuals	558	108	101	97	68	90	94
Positive individuals (%)	217 (38.9%)	58 (53.7%)	36 (35.6%)	21 (21.6%)	11 (16.2%)	63 (70.0%)	28 (29.8%)
Ct (min, max)	26.2 (10, 34)	27.9 (14, 33)	27.7 (11, 34)	24.4 (15, 32)	16.2 (10, 25)	27.2 (14, 32)	23.4 (13, 32)
MERS genomes (>90% complete)	42	8	6	8	4	4	12

Hassan, EID 2024

Full genome phylogenies of 2023-MERS-CoV, clade B5 sublineages

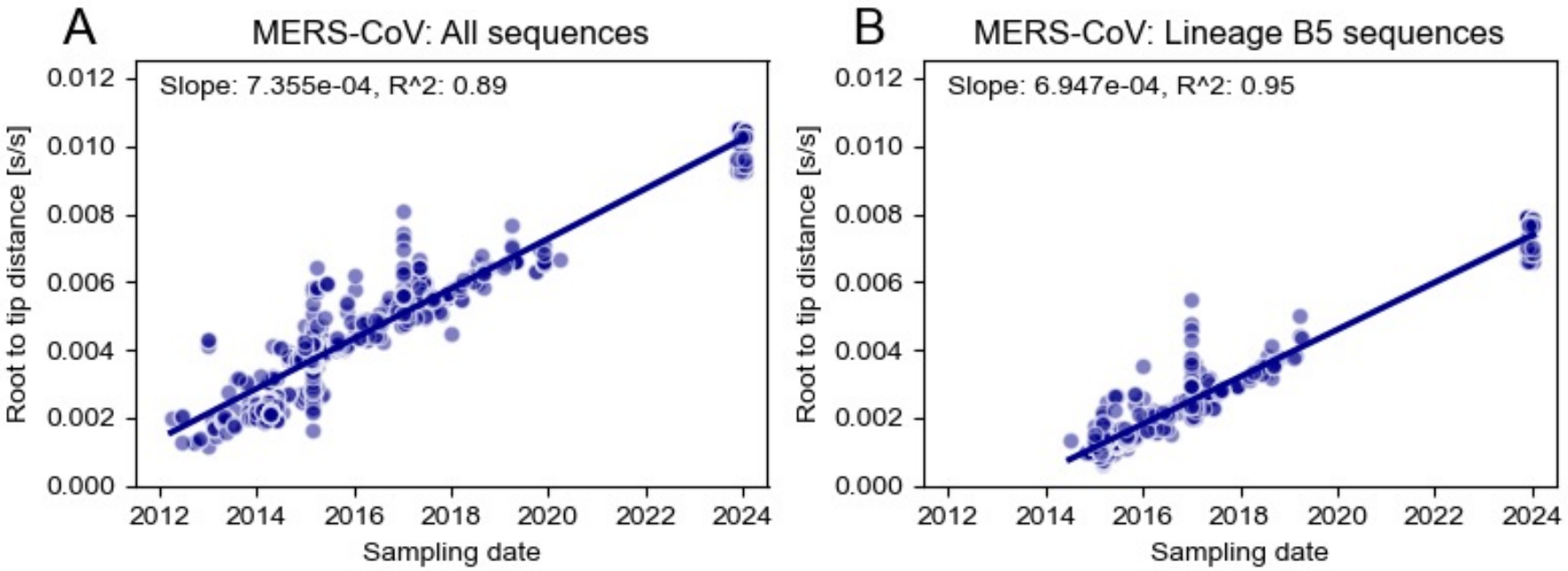


Hassan, EID 2024



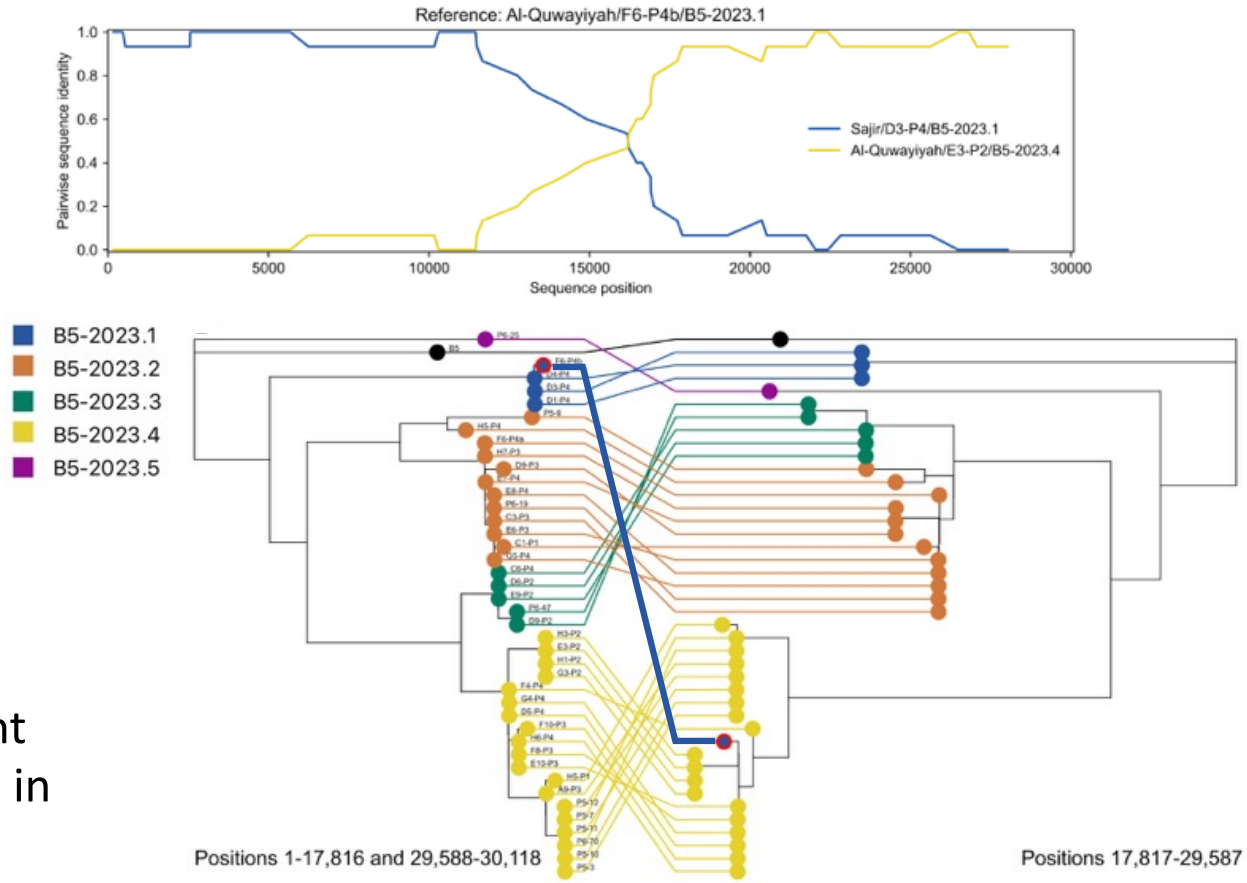
Hassan, EID 2024

Constant apparent substitution rate



Hassan, EID 2024

Recombination among B5 sublineages

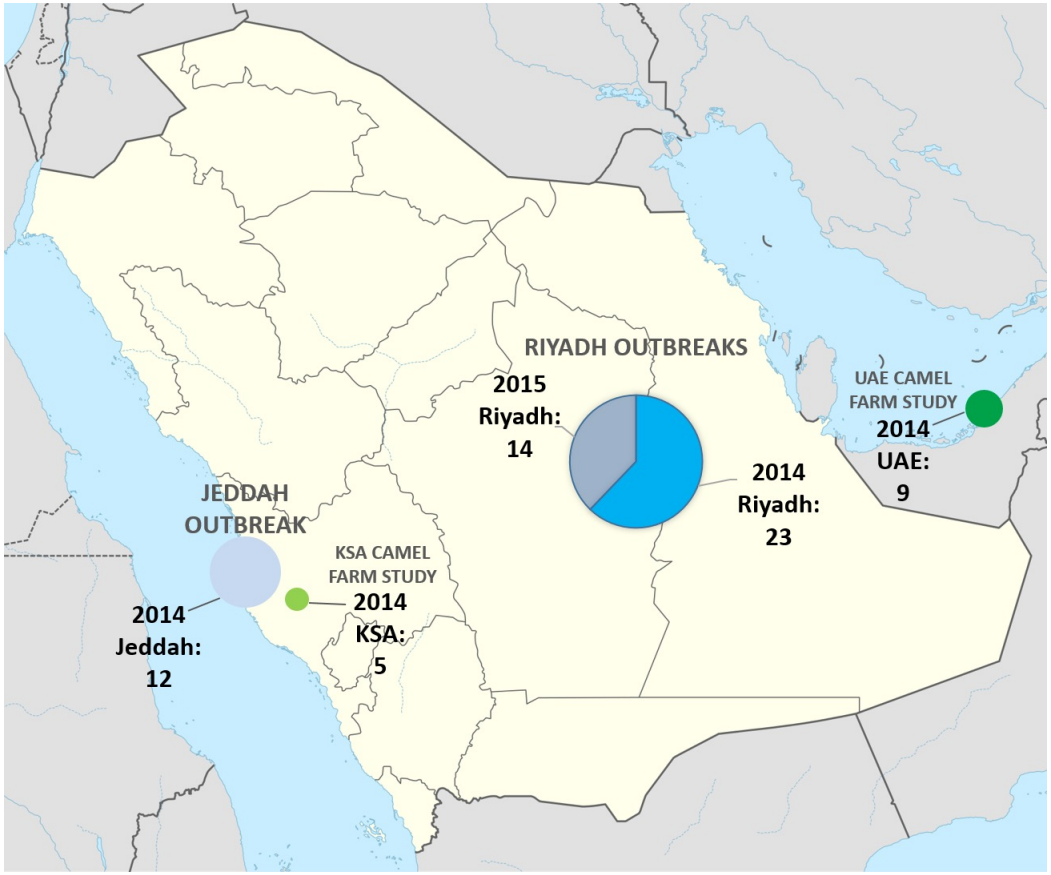


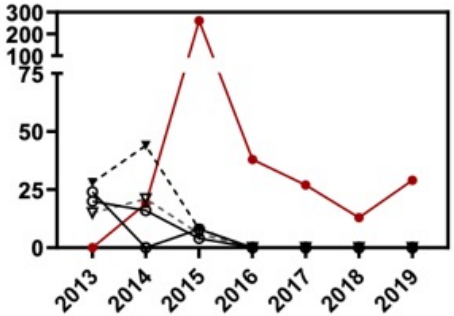
Recombination requires frequent double infection in camels.

Hassan, EID 2024

Lineage 5, phenotype

MERS CoV isolates from the time of lineage sweep (2014-2015)





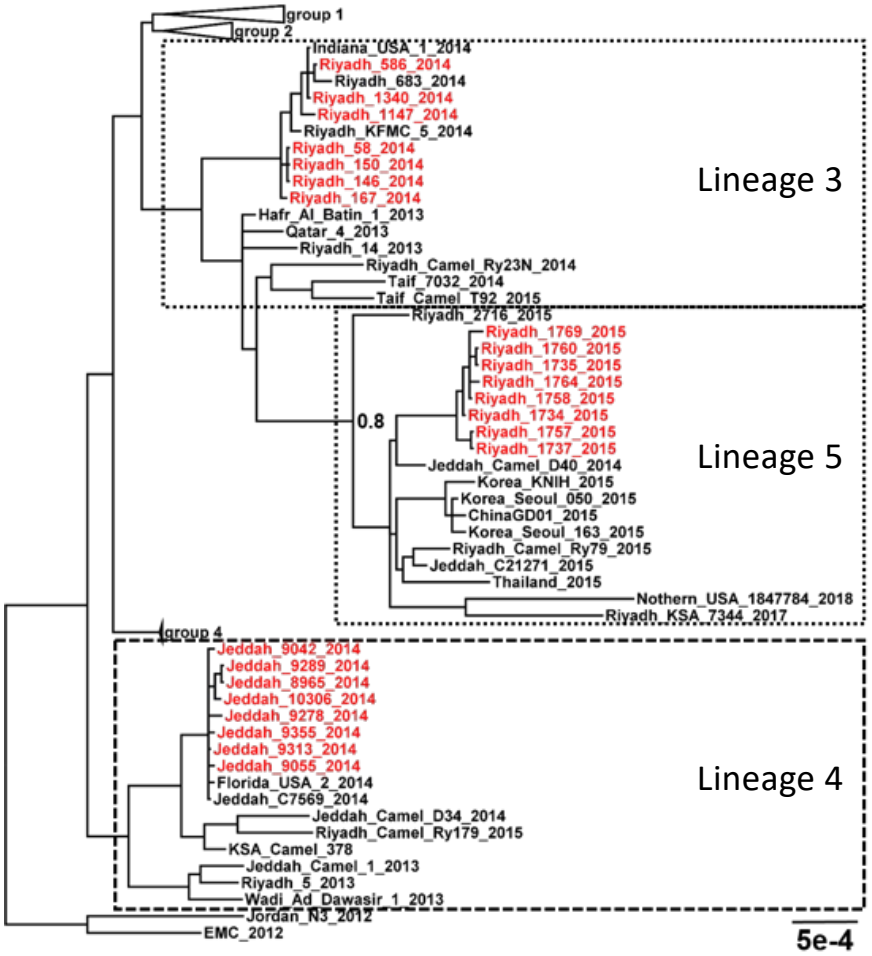
> 3000 samples received (Mar. 2014 – Nov. 2015)

137 isolation trials
64 successfull isolations

39 isolates stocked and deep sequenced

Muth et al., 2016 and Schröder et al., 2021

Full genome phylogeny of human-derived isolates, KSA



Recombination parent

Recombination descendant

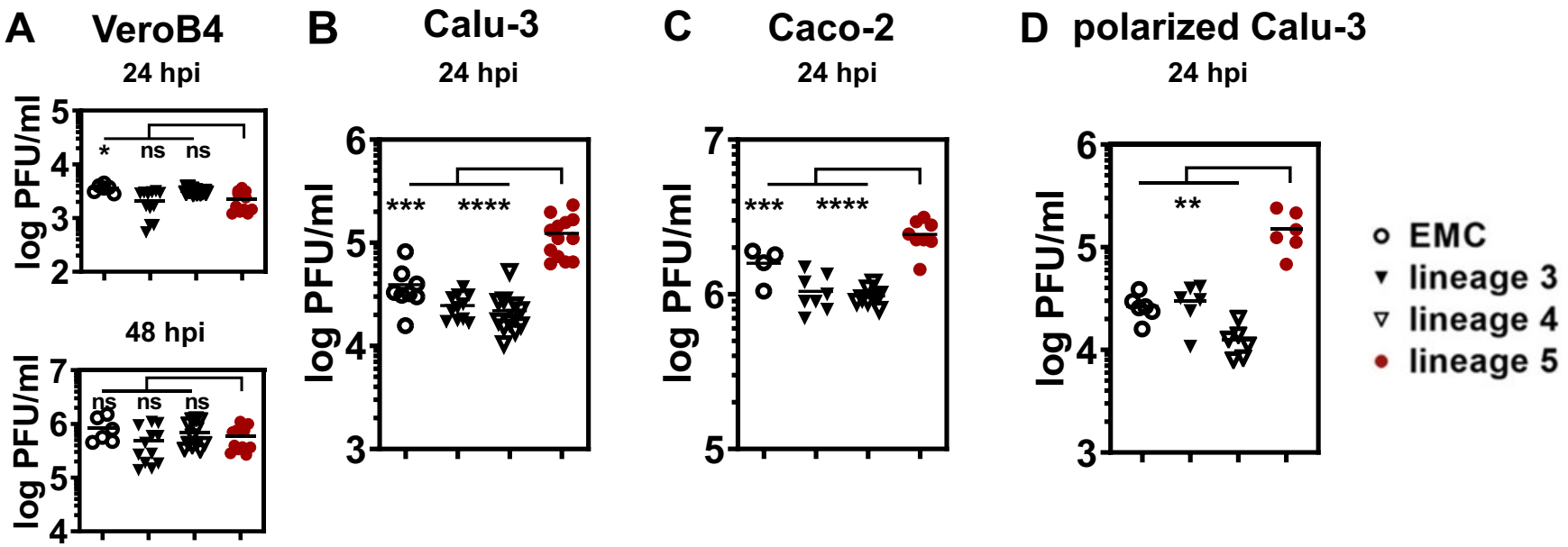
Recombination parent

Live virus isolates generated

Lineage 3: N = 7
Lineage 4: N = 8
Lineage 5: N = 8

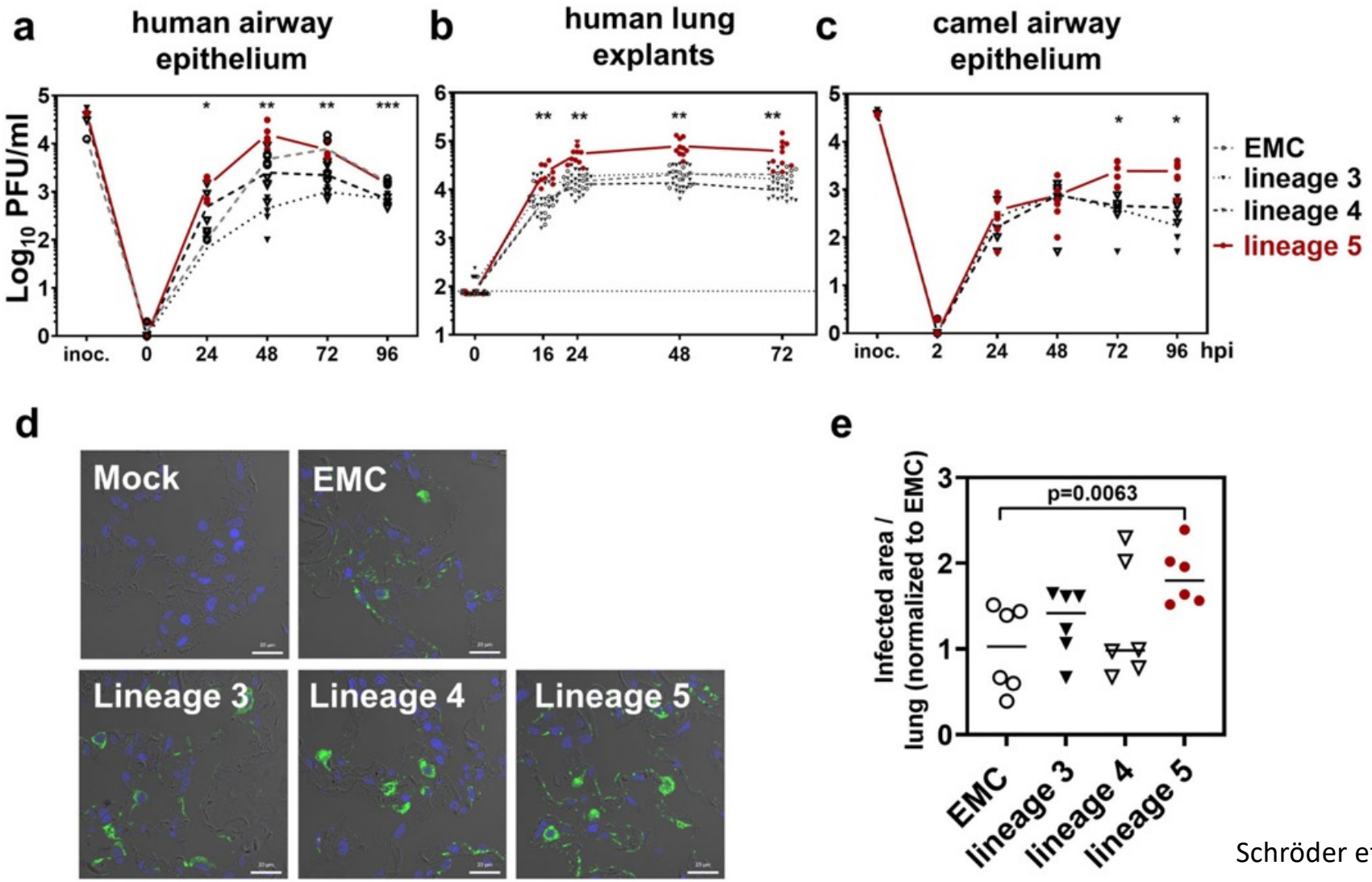
Schröder et al., 2021

Growth kinetics



3-4 reps,
2-4 viruses each

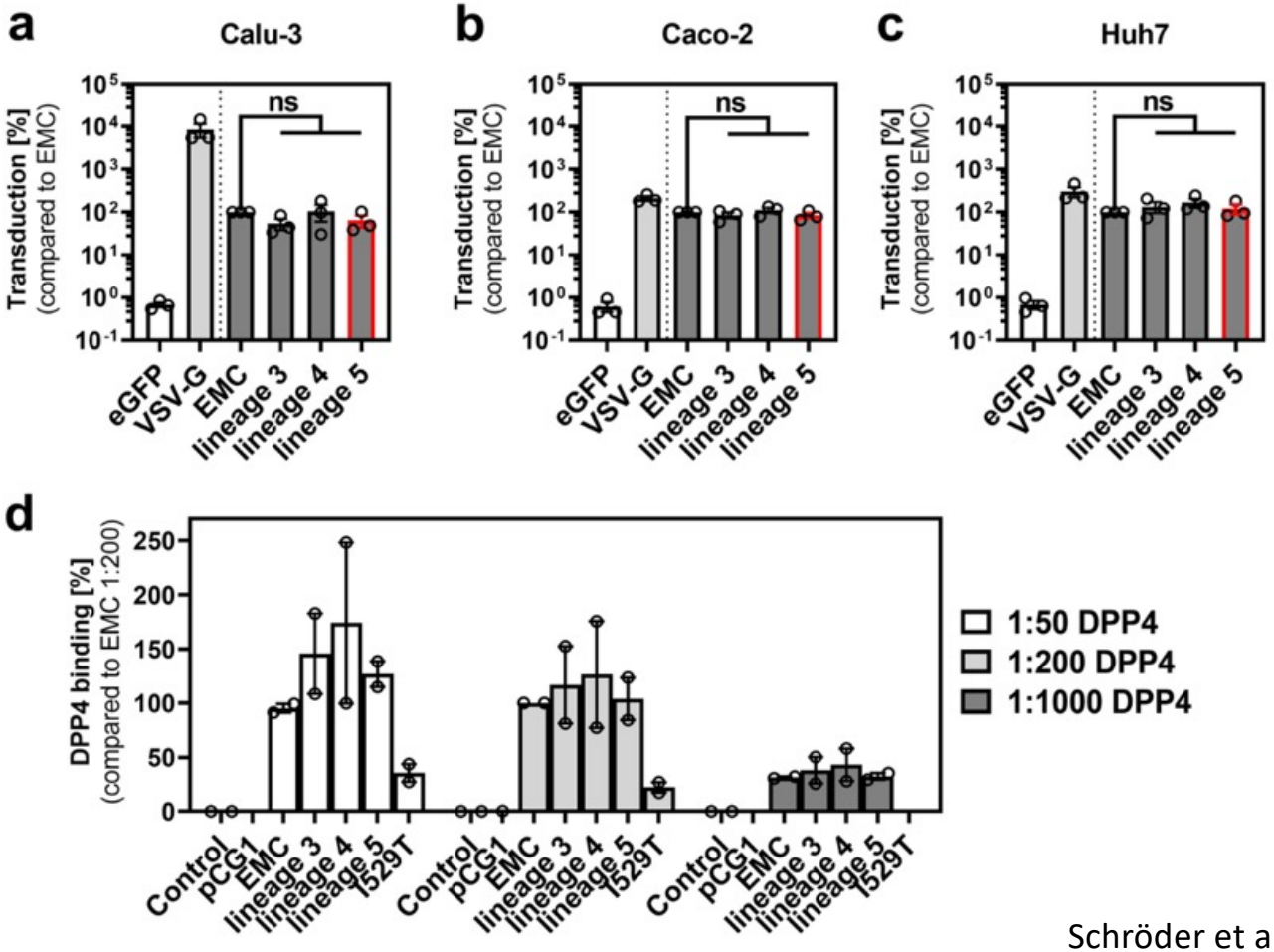
Schröder et al., 2021



Schröder et al., 2021

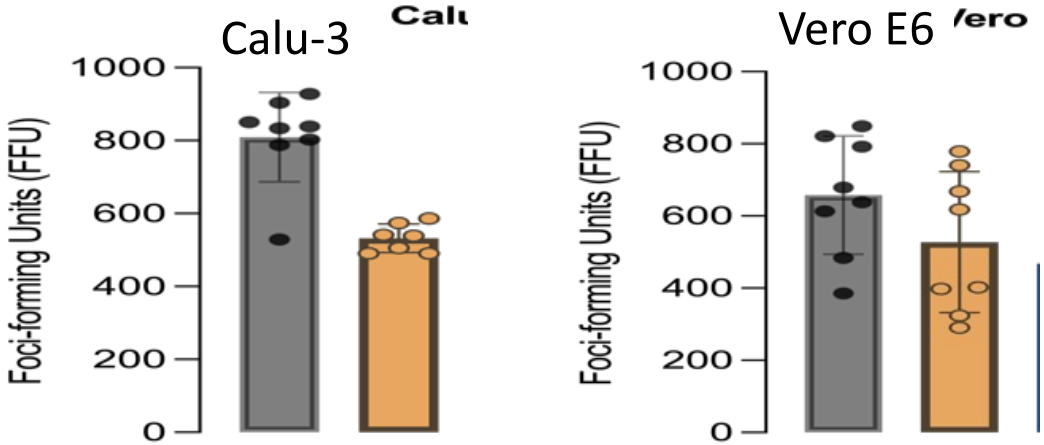
No difference in entry (VSV pseudotypes) and receptor binding (FACS-based assay)

Data from S Pöhlmann lab



Schröder et al., 2021

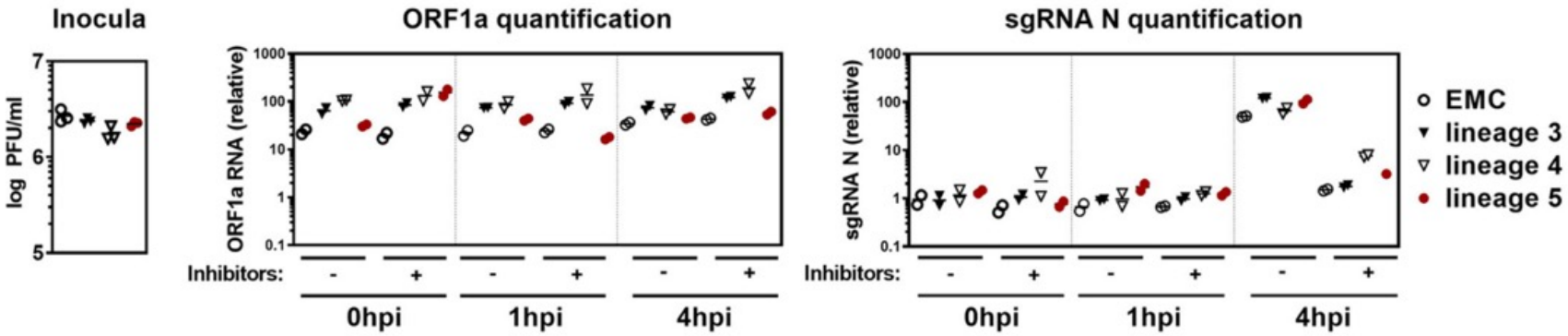
Entry of MERS-CoV lineage 5, recent lineages



*Inoculation dose: 500 FFU in Vero E6-TMPRSS2

Rodon et al., unpublished

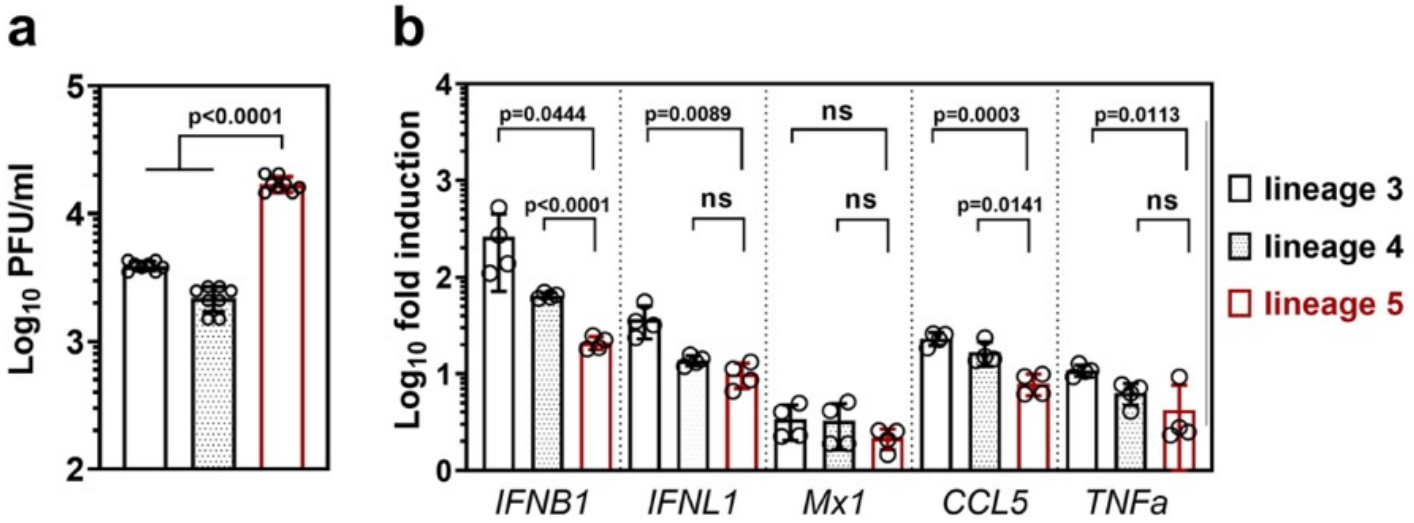
No difference in entry using full virus



Adsorb virus at 4°C, shift to 37°C, read genome or early (N) transcript RNA
Experiment with/wo inhibitor mix acting against TMPRSS2, cathepsin L, clathrin

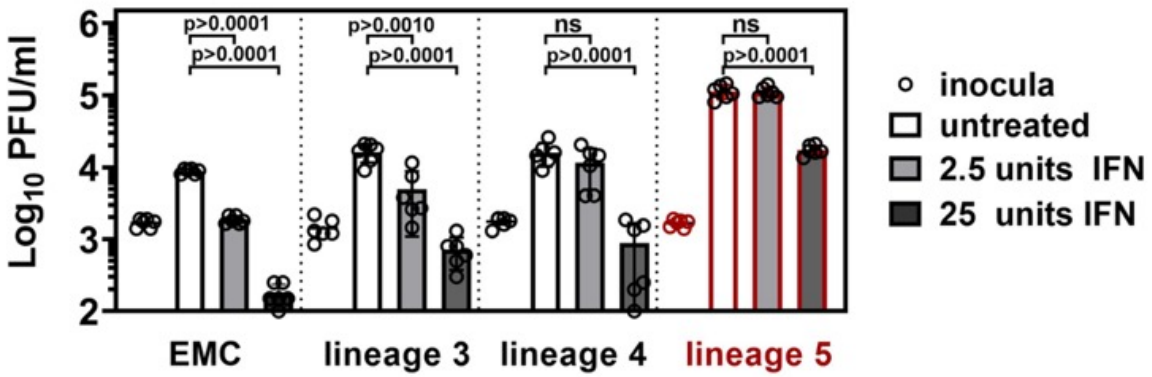
Schröder et al., 2021

Lineage 5 suppresses cytokine induction more effectively than parental lineages



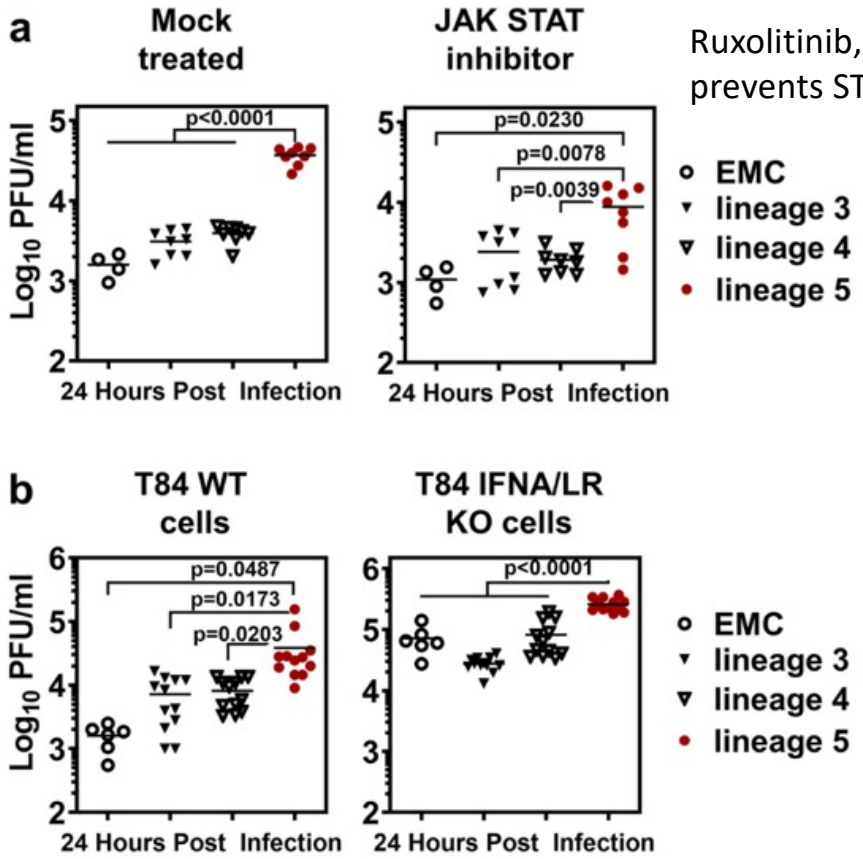
Schröder et al., 2021

Lineage 5 is more resilient against IFN



Schröder et al., 2021

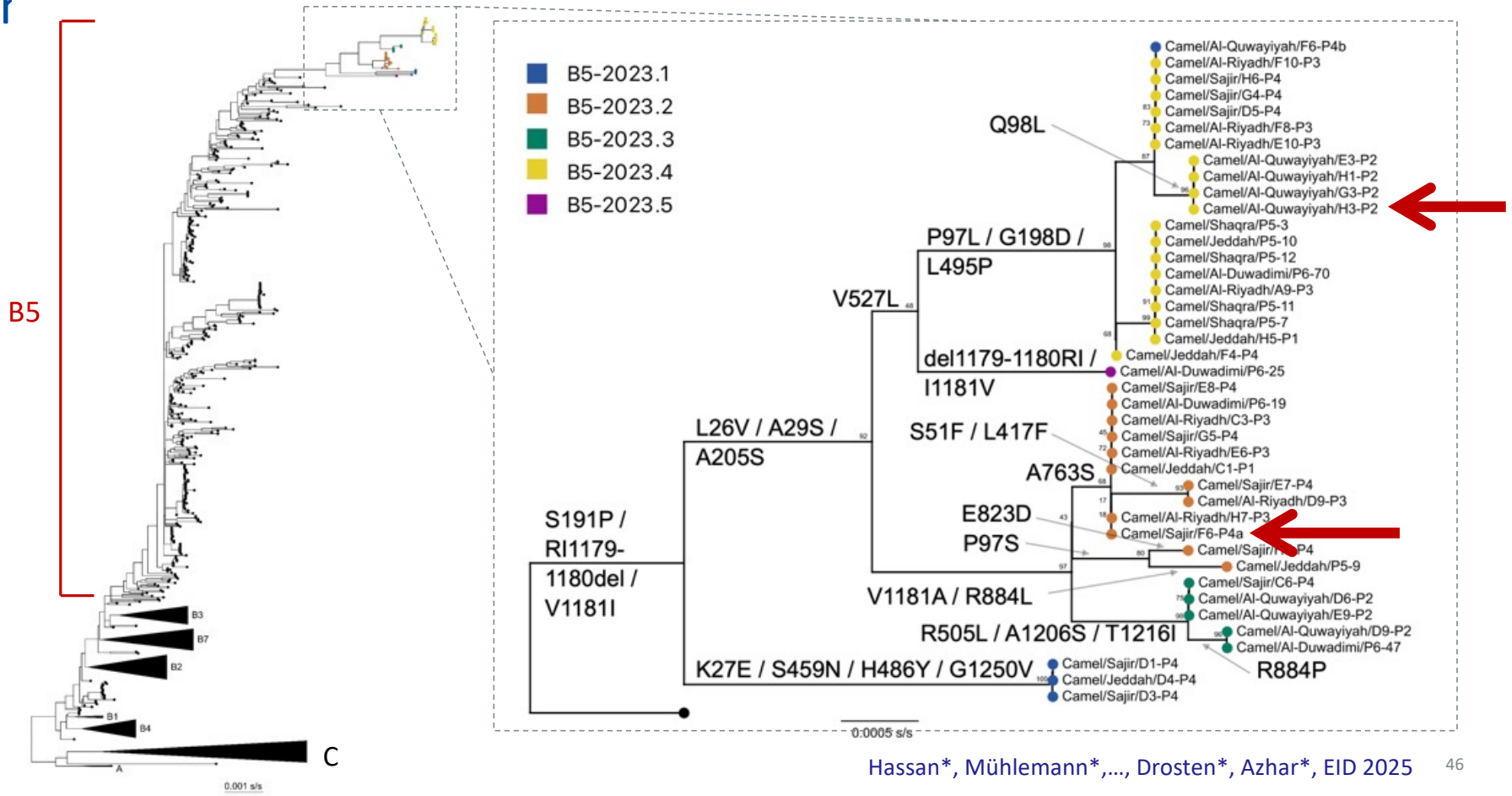
Resilience is based on IFN type I receptor availability



Ruxolitinib, JAK kinase inhibitor, prevents STAT-1/2 phosporylation

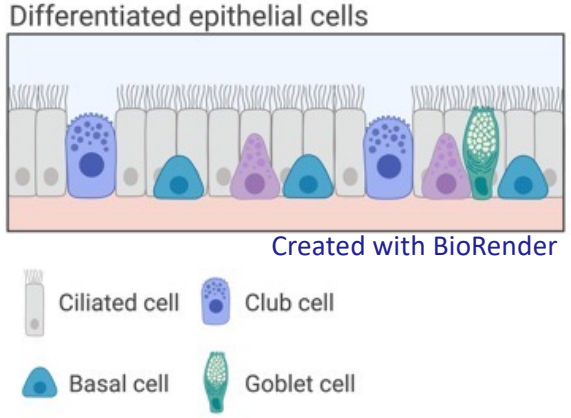
Schröder et al., 2021

Rescue of postpandemic lineage 5 viruses by reverse genetics (TAR cloning)

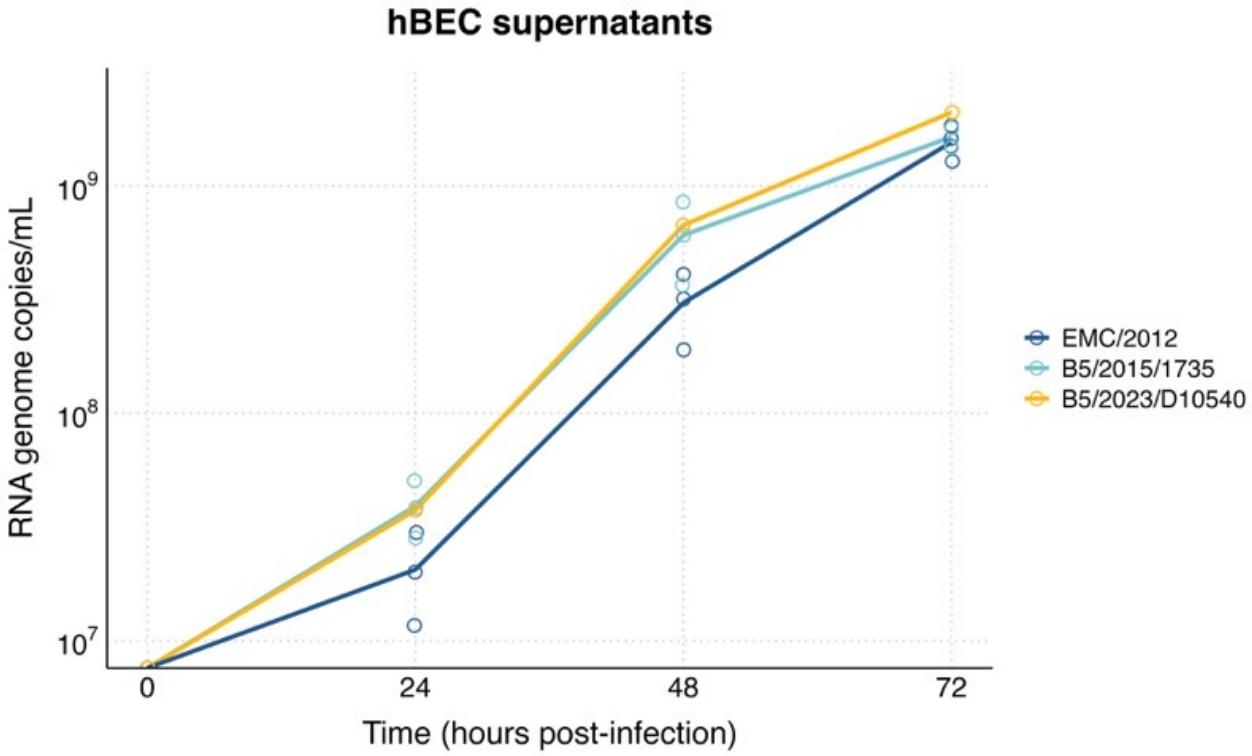


Hassan*, Mühlemann*,..., Drosten*, Azhar*, EID 2025

MERS-CoV lineage B5 strains from 2023-2024 replicate efficiently in hBECs

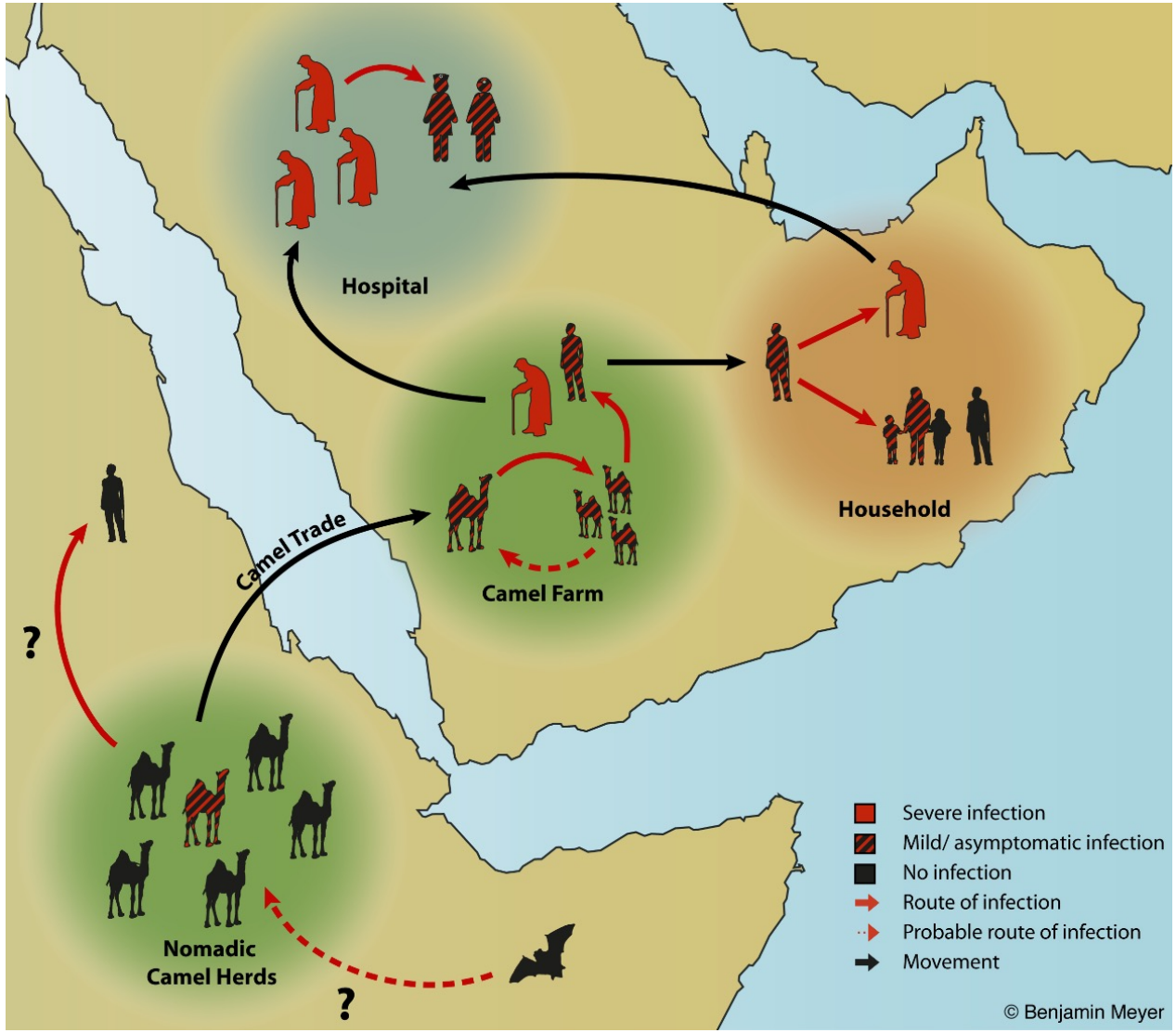


- **No obvious change of replication in postpandemic MERS-CoV lineage 5 (differentiated human bronchial epithelial model)**

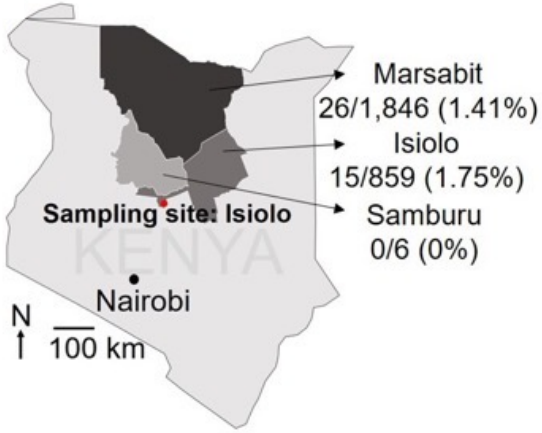


MERS lineage 5 shows an intrinsically increased fitness, attributable to increased suppression and evasion of IFN response

Enzootic virus in Africa



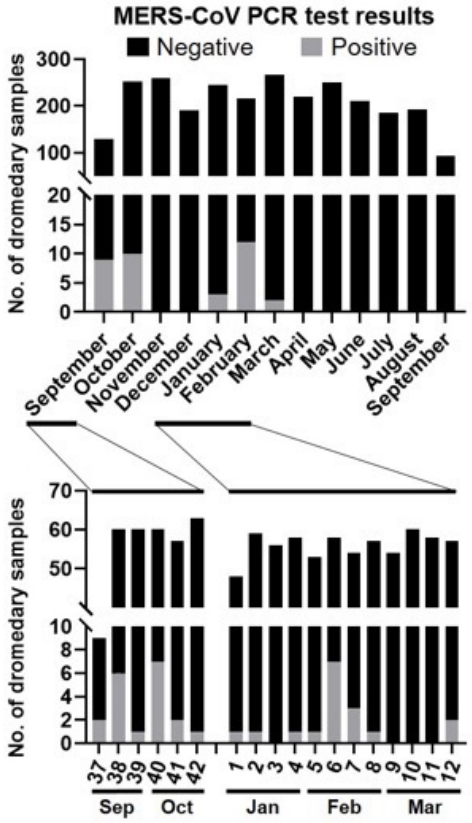
Biphasic MERS-CoV detection in dromedaries in Northern Kenya



Daily camel sampling over 12 months in abattoir hub (Isiolo)

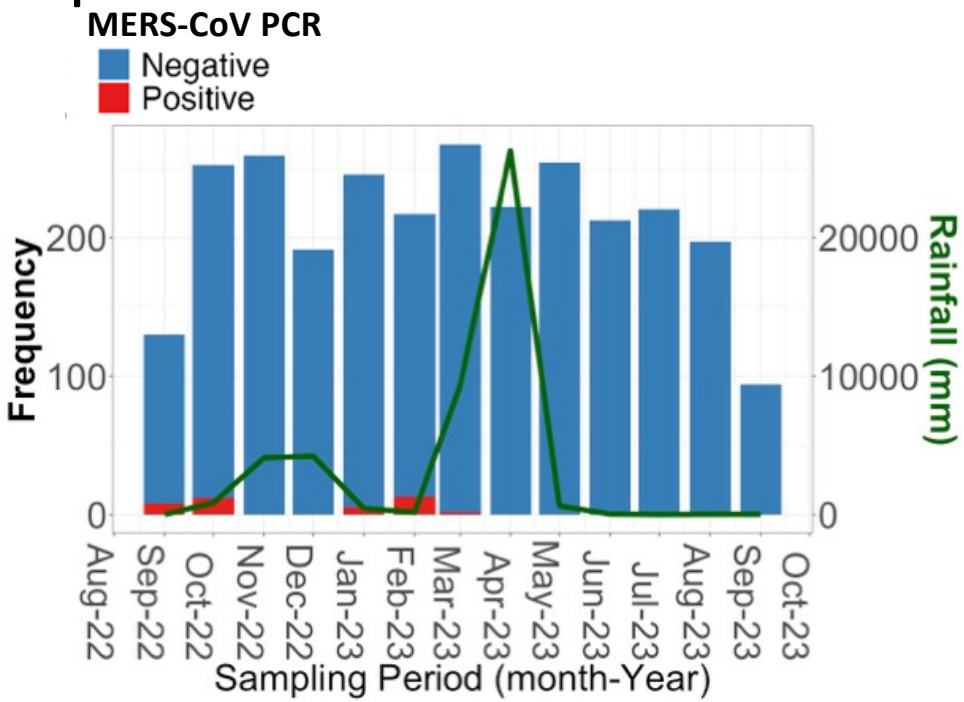
n=36/2711 MERS-CoV RNA positive

Peak MERS-CoV RNA detections in October and February



Ogoti et al., 2024

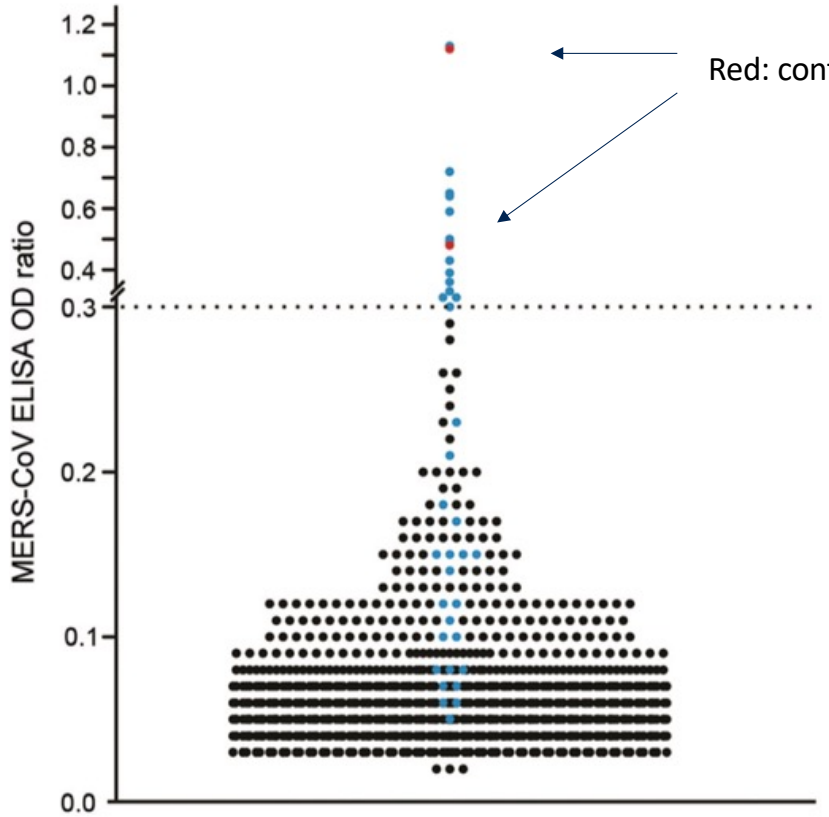
Infection incidence in dromedaries correlates with rainfall patterns



MERS-CoV cases vs rainfall (r_s = -0.5): negative correlation

Potential role of reduction of accessible waterholes during dry season (increase of contact rate around water holes; shedding of virus into surface water)

Ogoti et al., 2024



Is there really no MERS in humans?

Early evidence of human MERS infection in Africa (Kenya)

Table. Seropositivity for Middle East respiratory syndrome coronavirus in samples from humans in Kenya, 2013–2014*

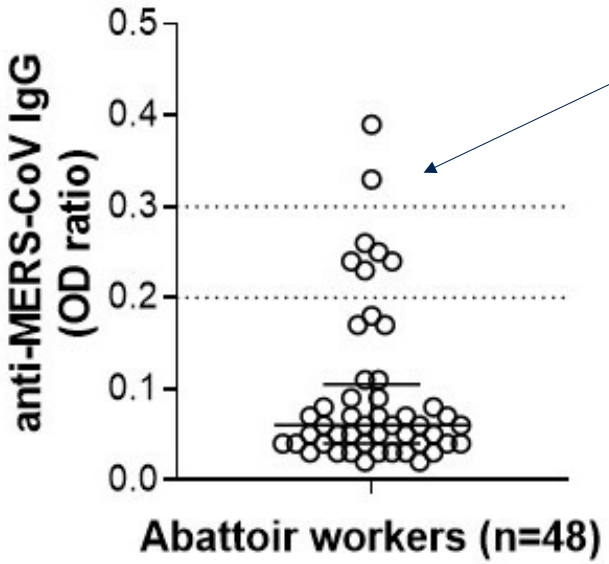
County	No. samples tested	No. (%) positive by rELISA	No. (%) positive by rELISA and with ≥50% plaque reduction at 1:20 dilution
Garissa	559	4 (0.72)	0
Tana River	563	12 (2.13)	2 (0.36)
Total	1,122	16 (1.43)	2 (0.18)

*rELISA, recombinant ELISA.

Liljander et al., 2016

MERS-CoV seropositivity in camel-exposed abattoir workers in Northern Kenya (ongoing field studies)

MERS-CoV S1 ELISA (commercial)



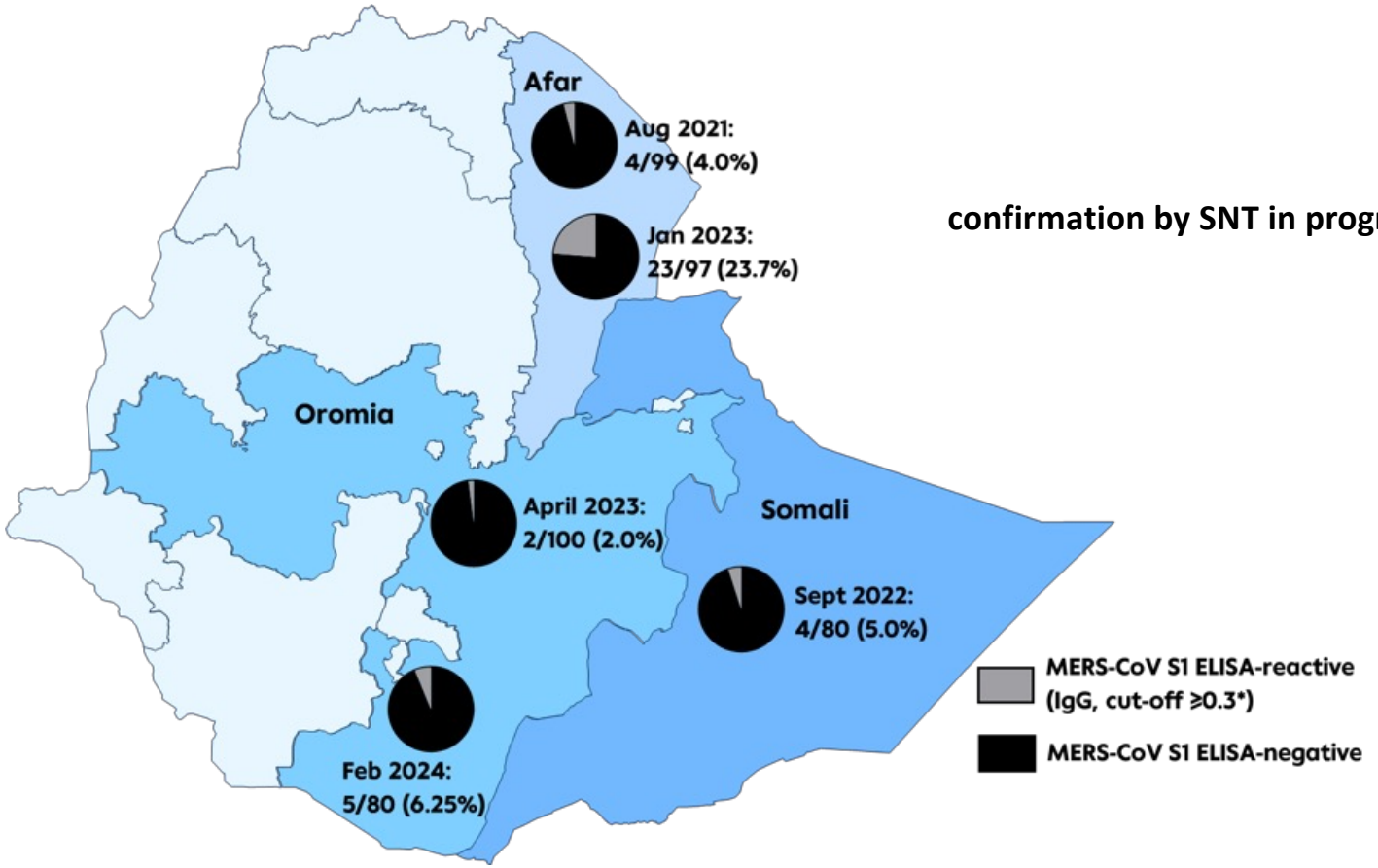
confirmed by serum neutralization test

7 of 48 camel-exposed abattoir workers show elevated anti-MERS-CoV S1 IgG reponse (ELISA)

Confirmation by SNT in 1 of 7 cases

Ogoti et al., 2024

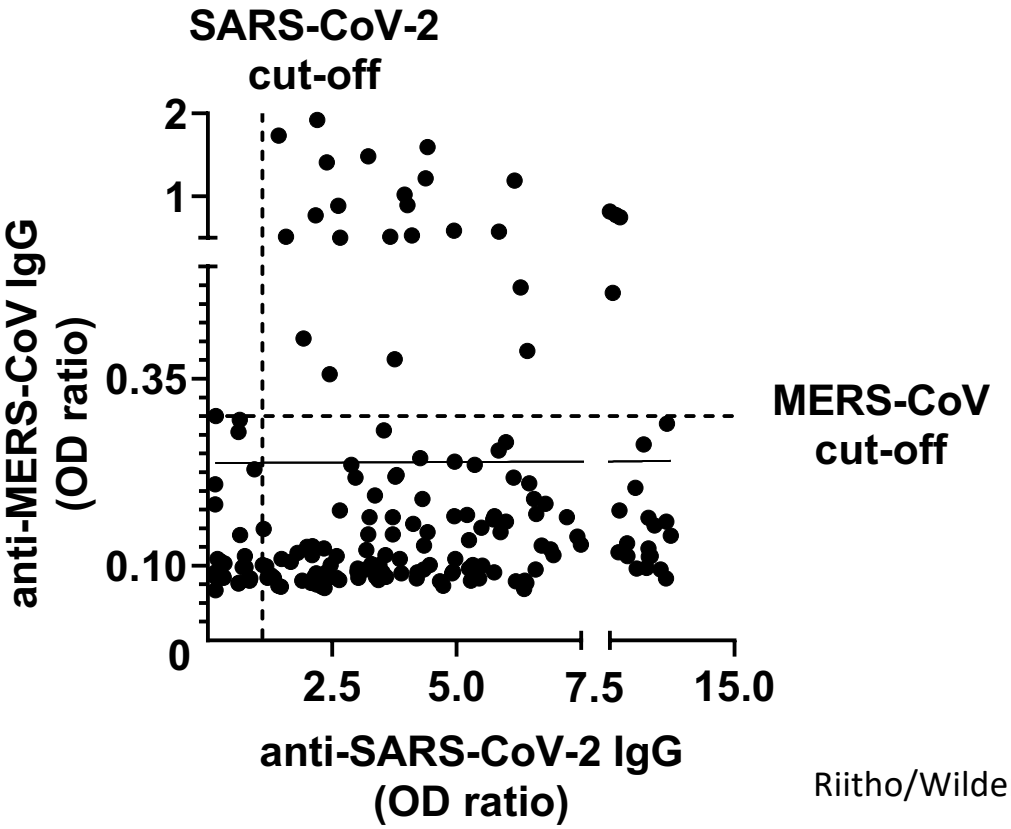
Human seroprevalence study in Ethiopia 2021-2024 (ELISA-based)



confirmation by SNT in progress…

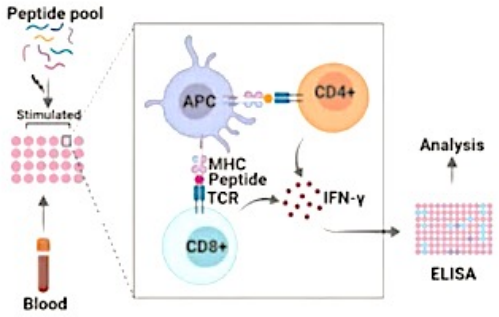
Riitho/Wildemann et al. unpublished

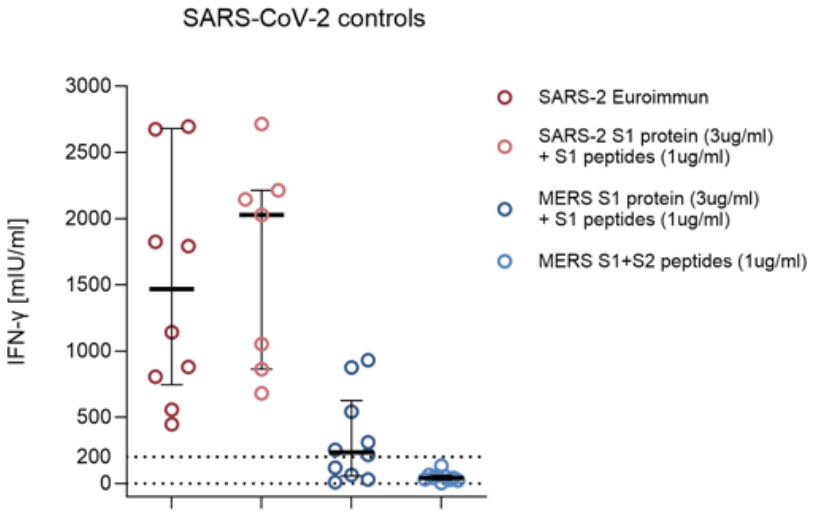
No apparent cross-reactivity with SARS-CoV-2 antibodies



Riitho/Wildemann et al. unpublished

IFN-gamma release assay (IGRA) for MERS-CoV T cell activation testing under lab conditions close to the field





Negative: <100 mIU/mL

Borderline: 100-200 mIU/mL

Positive: >200 mIU/mL

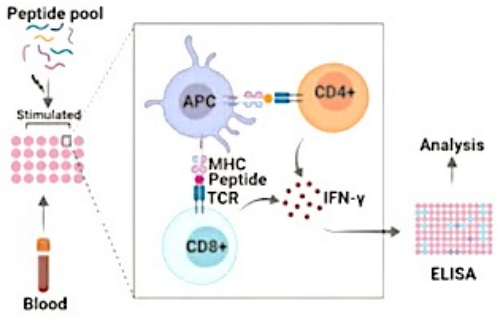
Two different peptide pools tested

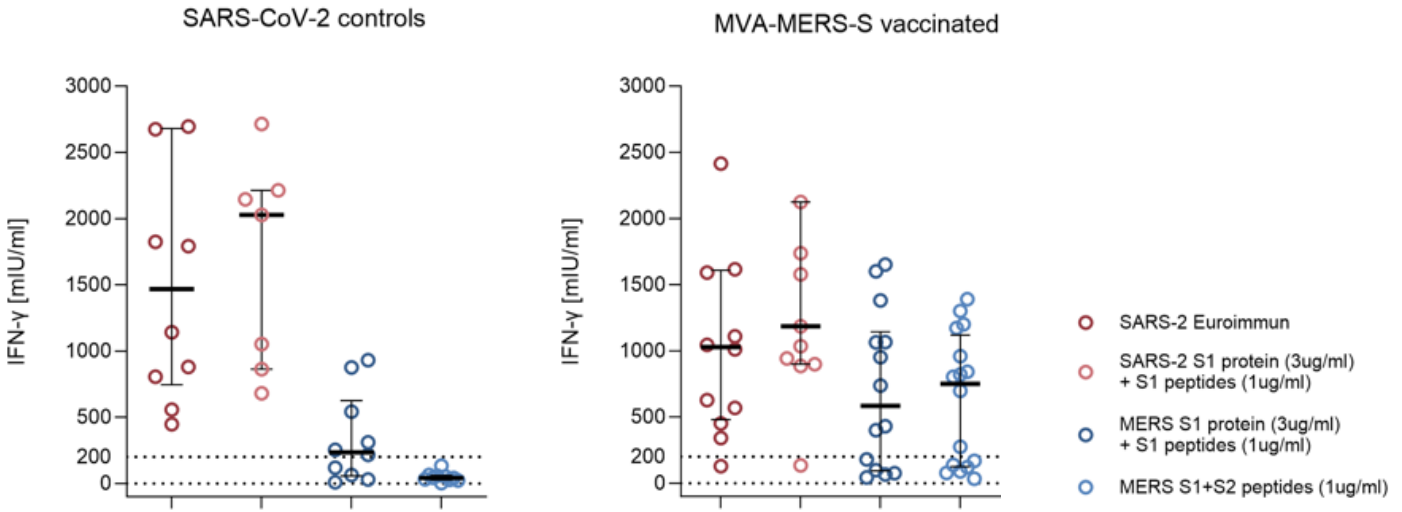


Victor Riitho and team at University of Nairobi

Riitho/Wildemann et al. unpublished

Validation of IFN-gamma release assay (IGRA) for MERS-CoV T cell activation:
SARS-CoV-2 infected vs. MVA-MERS-CoV-S human vaccinees





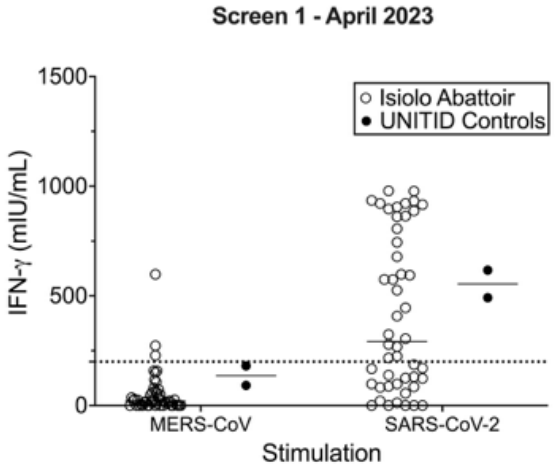
Two different peptide pools tested

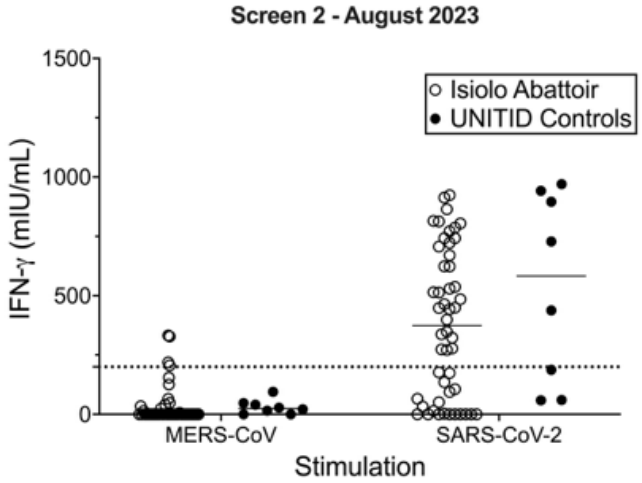
Collaboration with Leonie Mayer/ Marylyn Addo
University Medical Center Hamburg-Eppendorf

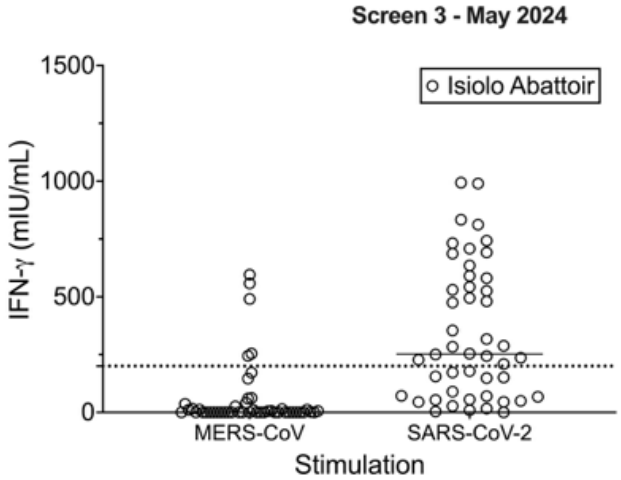
Riitho/Wildemann et al. unpublished

Negative: <100 mIU/mL
Borderline: 100-200 mIU/mL
Positive: >200 mIU/mL

MERS-CoV T cell responses in camel-exposed abattoir workers (n=48), Northern Kenya (2023/2024)







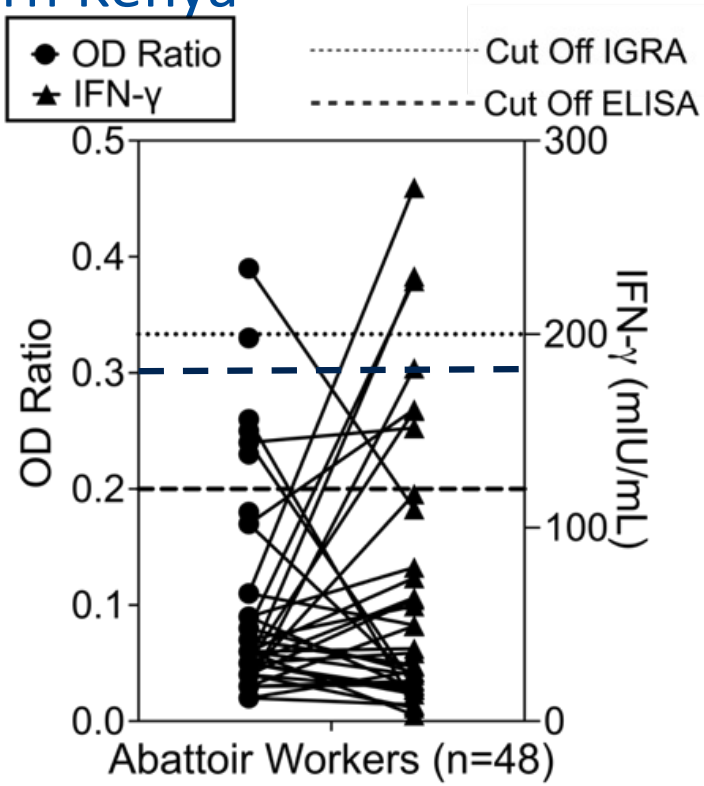
Abattoir Workers	Negative	Borderline	Positive	% Positive	n
MERS-CoV	40	5	3	6.25	48
SARS-CoV-2	13	7	28	58.33	48

Abattoir Workers	Negative	Borderline	Positive	% Positive	n
MERS-CoV	42	2	4	8.33	48
SARS-CoV-2	13	4	31	64.58	48

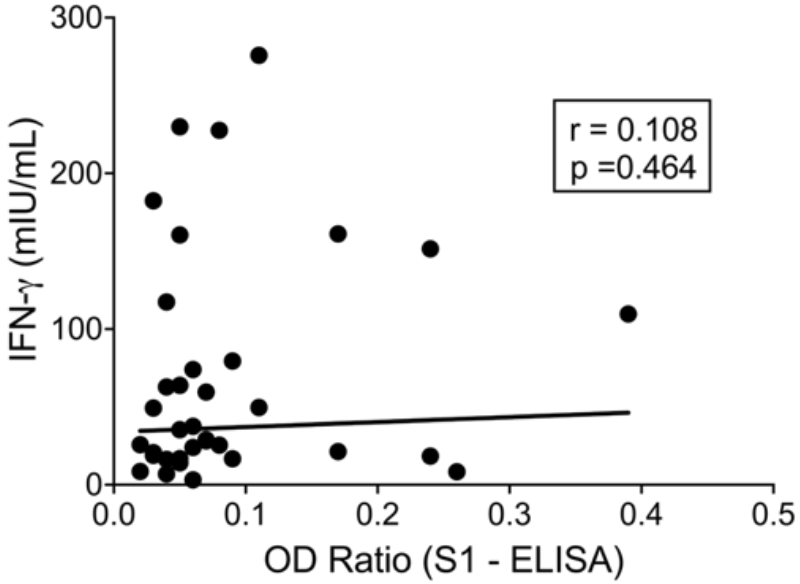
Abattoir Workers	Negative	Borderline	Positive	% Positive	n
MERS-CoV	41	2	5	10.42	48
SARS-CoV-2	14	5	29	60.42	48

Riitho/Wildemann et al. unpublished

MERS-CoV T cell response in camel-exposed abattoir workers in Northern Kenya



MERS IGRA and IgG responses are not correleated



Riitho/Wildemann et al. unpublished

Genomes and function





Camels imported in Jeddah harbor

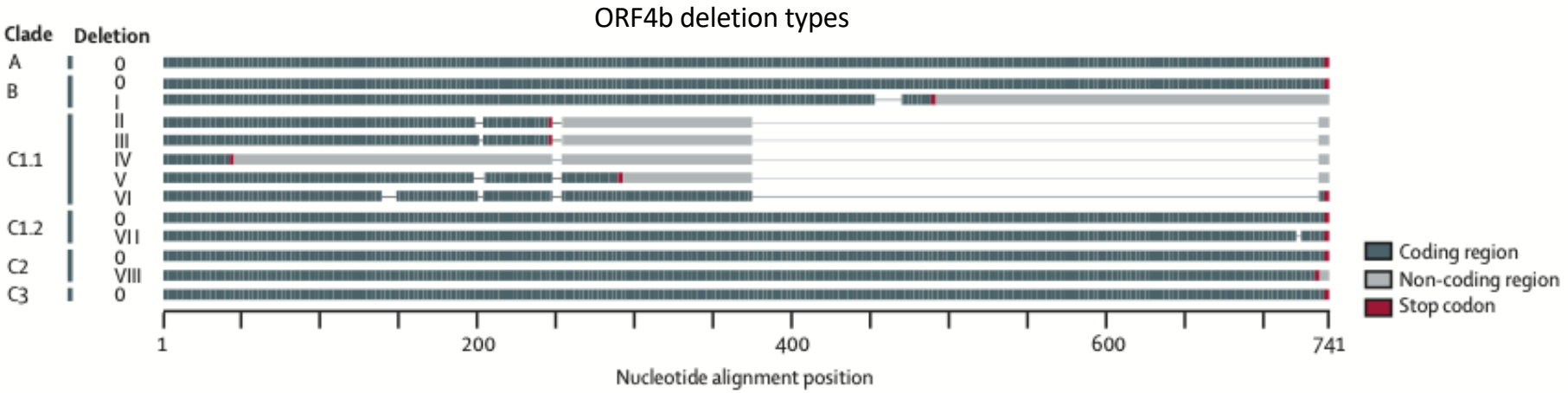
Origin	N	N pos	Fract.	Clade
Sudan, Djibouti	1196	157	13%	All C
Local	472	224	47%	All B

African genotypes reach the Arabian Peninsula but are never detected in local camels sampled in the same geospatial context.

El-Kafrawy et al., 2020

Many African viruses, not Arabian viruses, have deletions in accessory ORFs.

Most in ORF4b.



El-Kafrawy et al., 2020

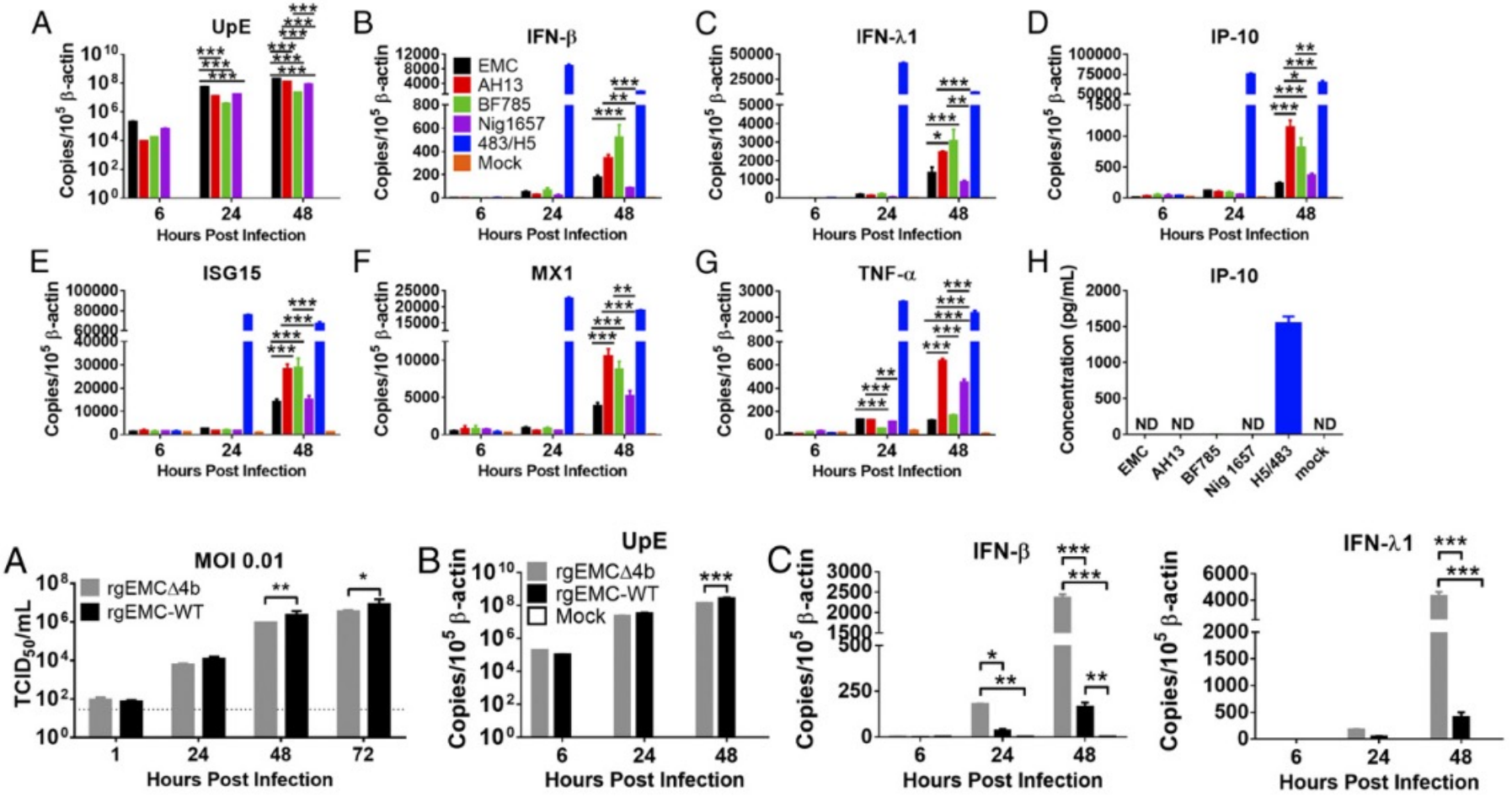
MERS-CoV genome

NLS = Nuclear localisation signal

PDE = Phosphodiesterase

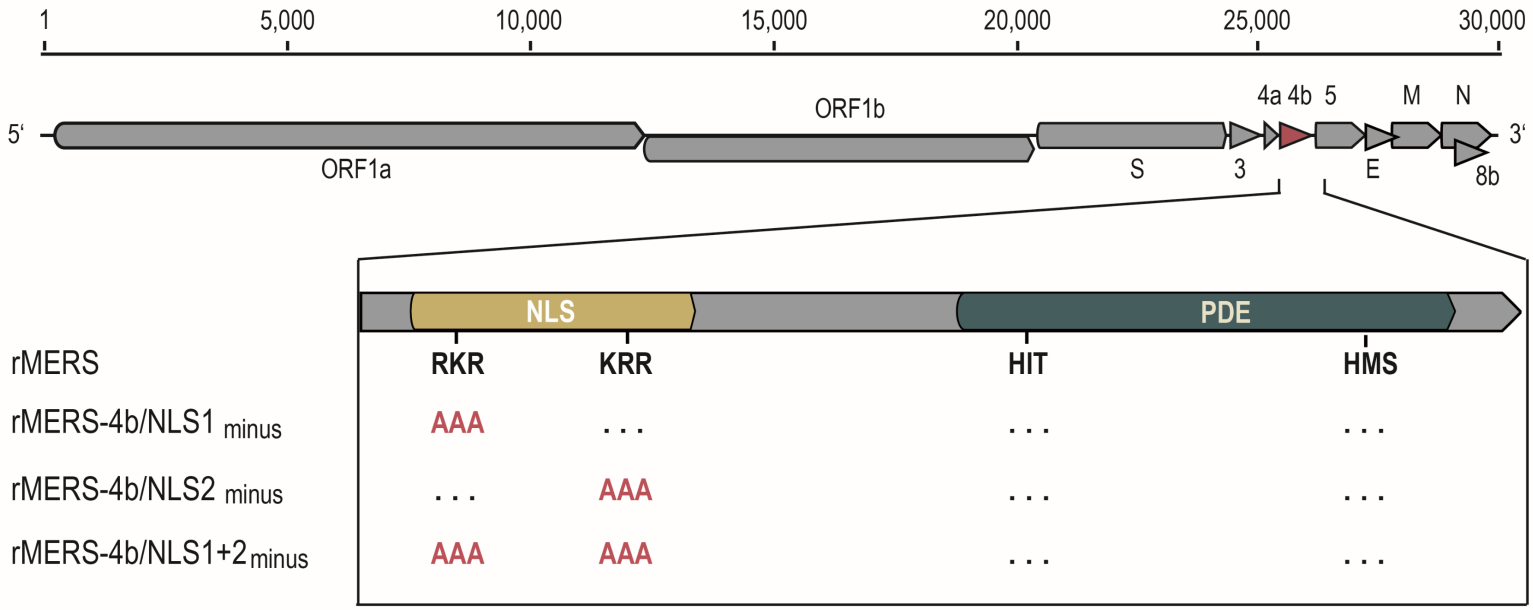
Viruses isolated from African camels

Eng. ORF4b deletion Full virus isolate

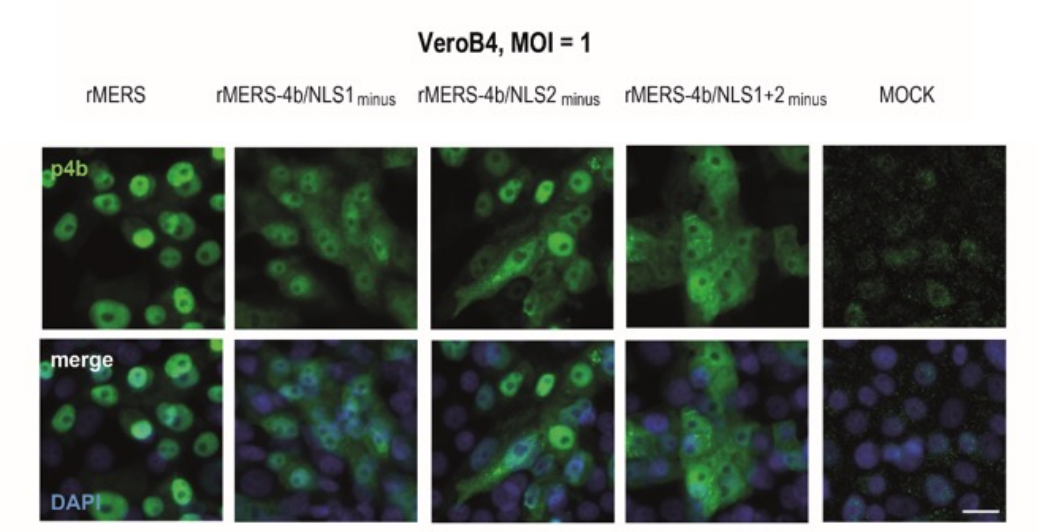


Chu, PNAS 2018

Recombinant MERS-CoV with ablated nuclear localisation signal (NLS)



Loss of 4b nuclear localization upon NLS ablation

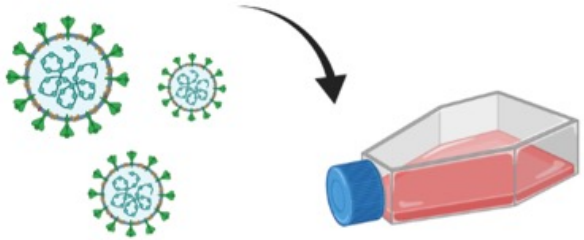


bar = 20 µm

Friedmann, unpublished

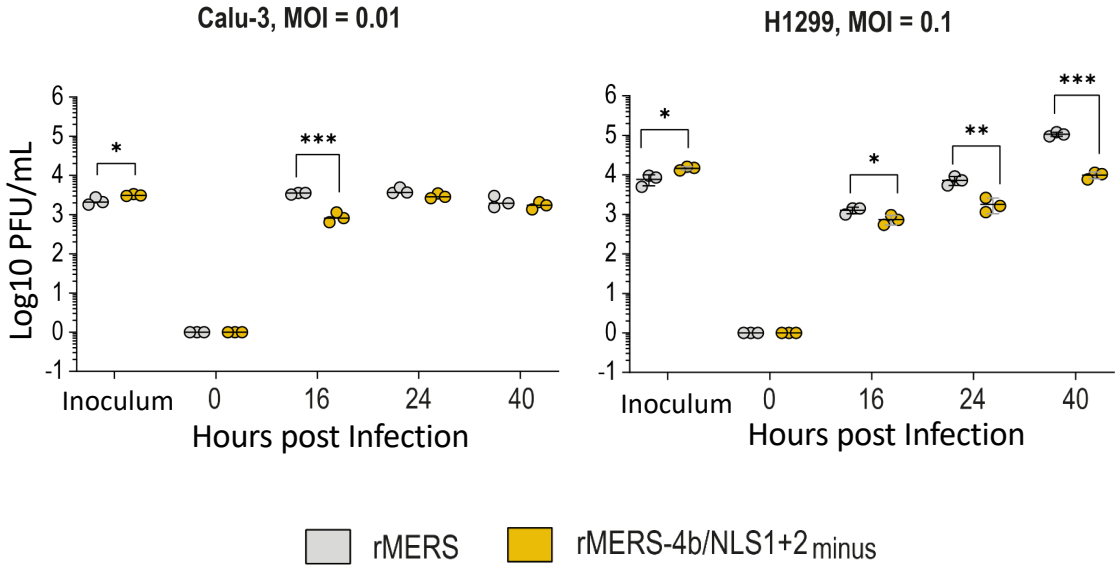
Reduction of replicative fitness with NLS inactivation

Growth in human lung cells



Calu-3
H1299

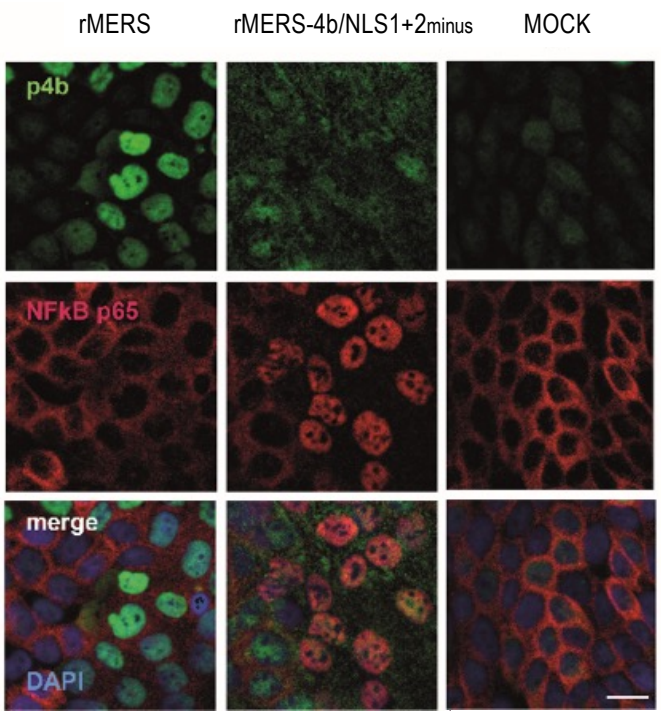
Illustriert mit Biorender.com



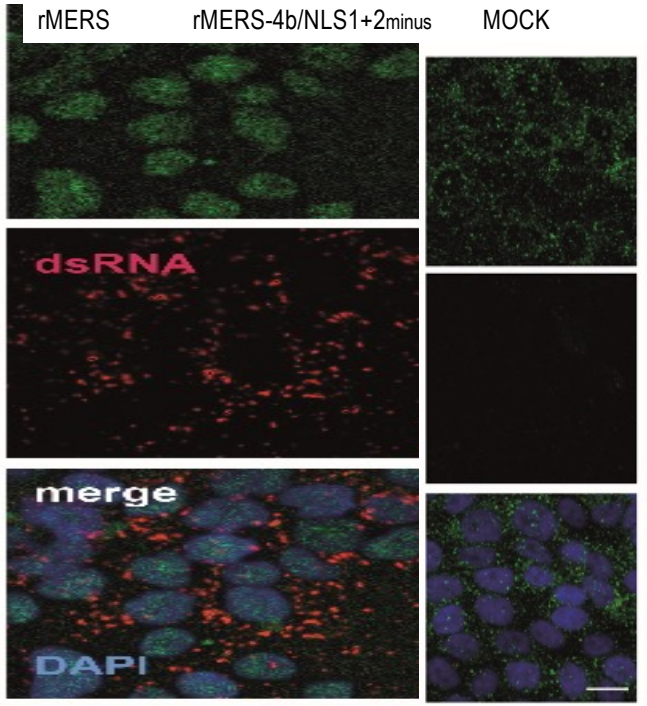
Friedmann, unpublished

Functional NLS blocks nuclear translocation of NFkB and IRF3

NFκB



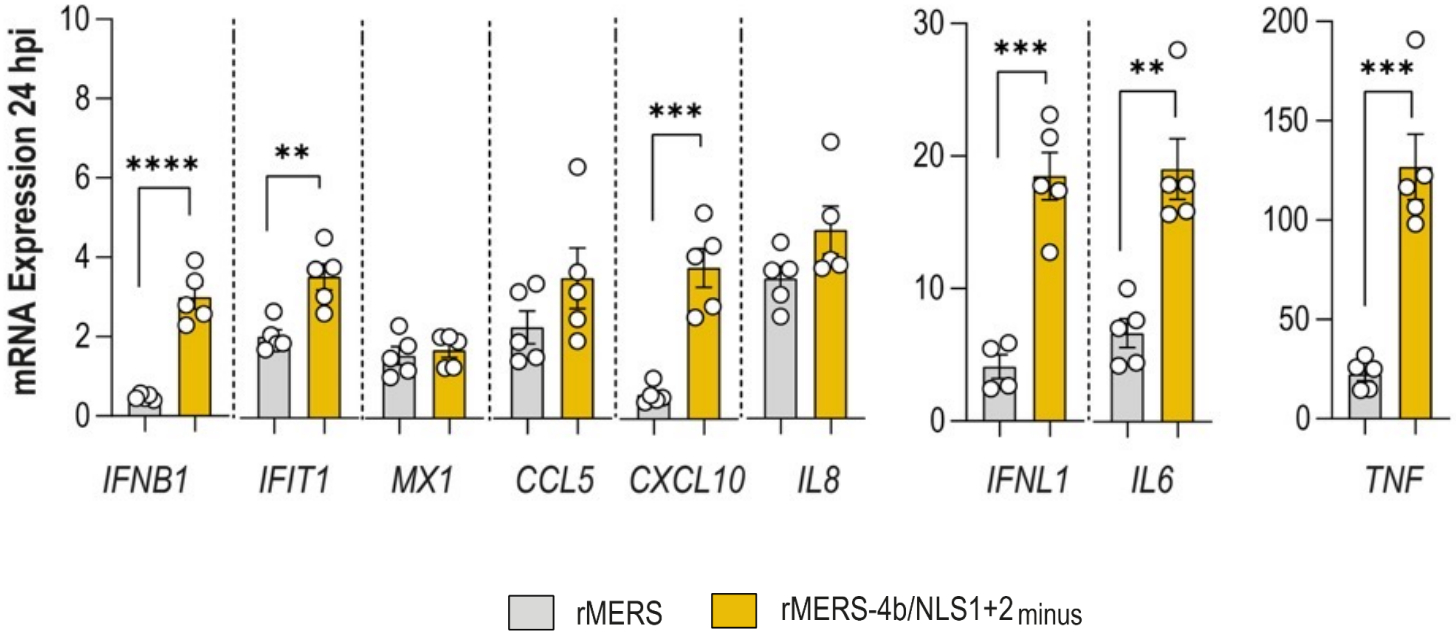
IRF3



Friedmann, unpublished

Calu-3

Loss of NLS increases cytokine and ISG expression

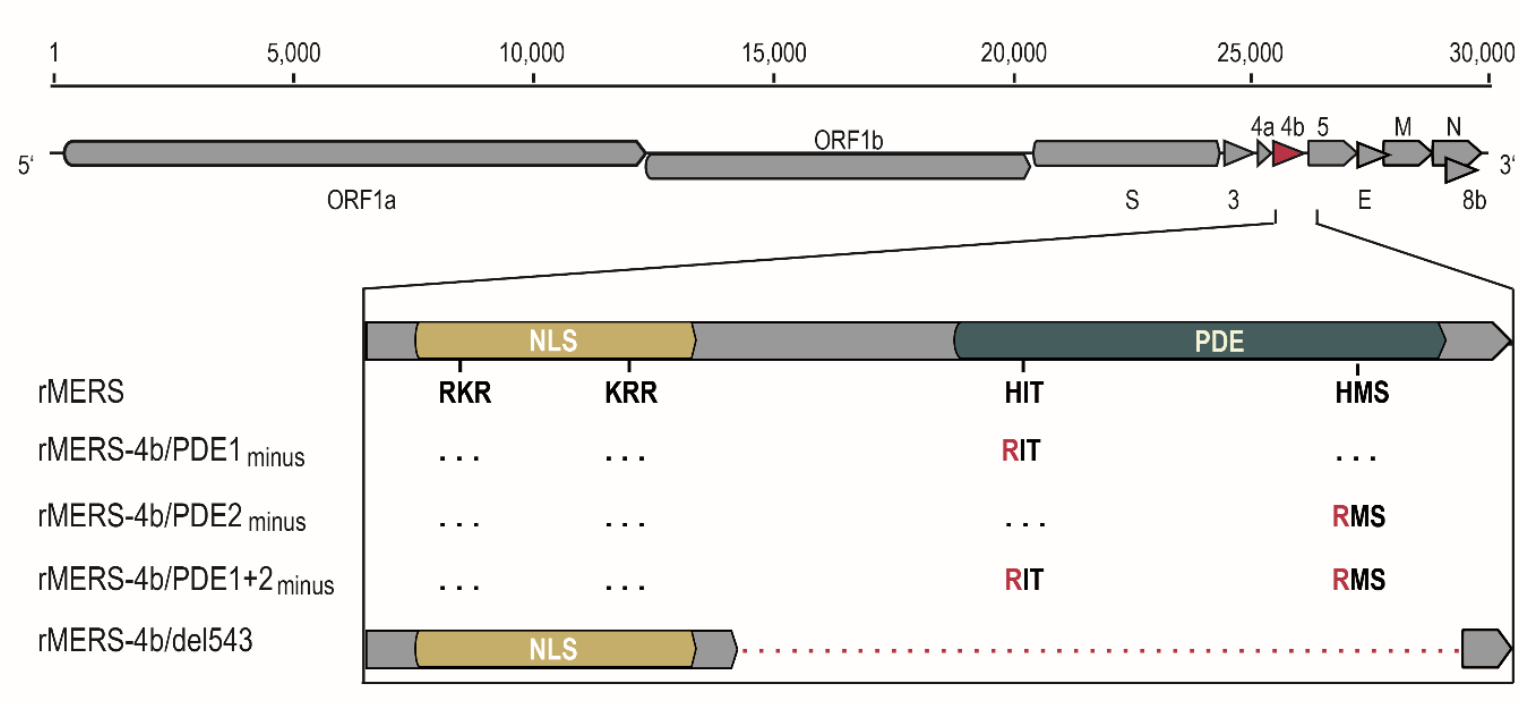


Calu-3

Friedmann, unpublished

…less obvious phenotype with deletion of phosphodiesterase domain

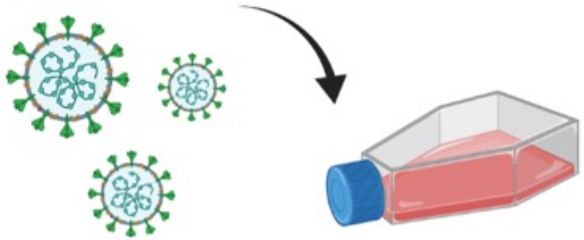
Recombinant MERS-CoV with ablated / deleted phosphodiesterase domain



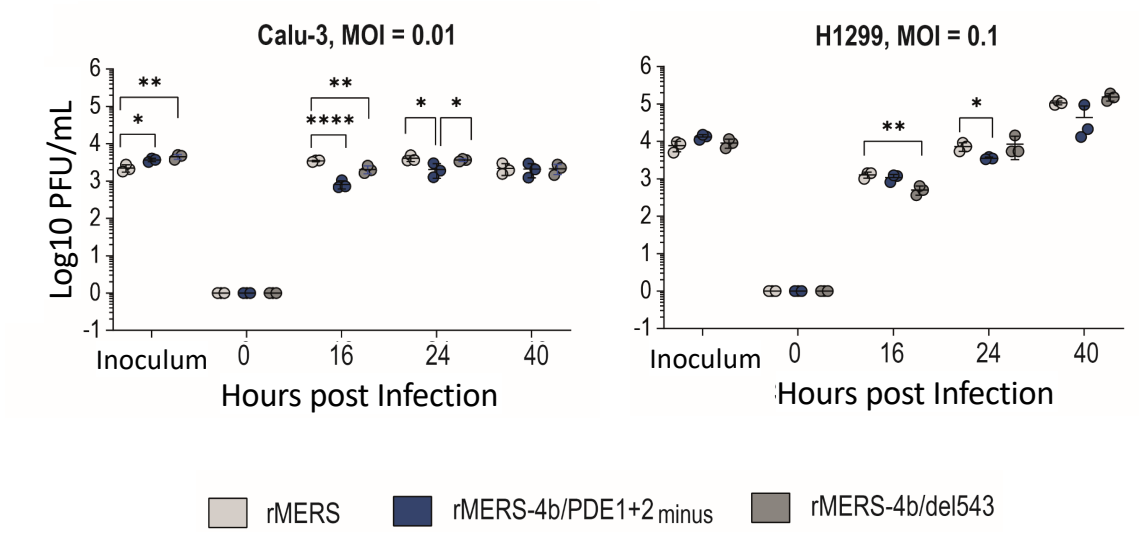
Friedmann, unpublished

Minor phenotypic change by deletion of PDE domain

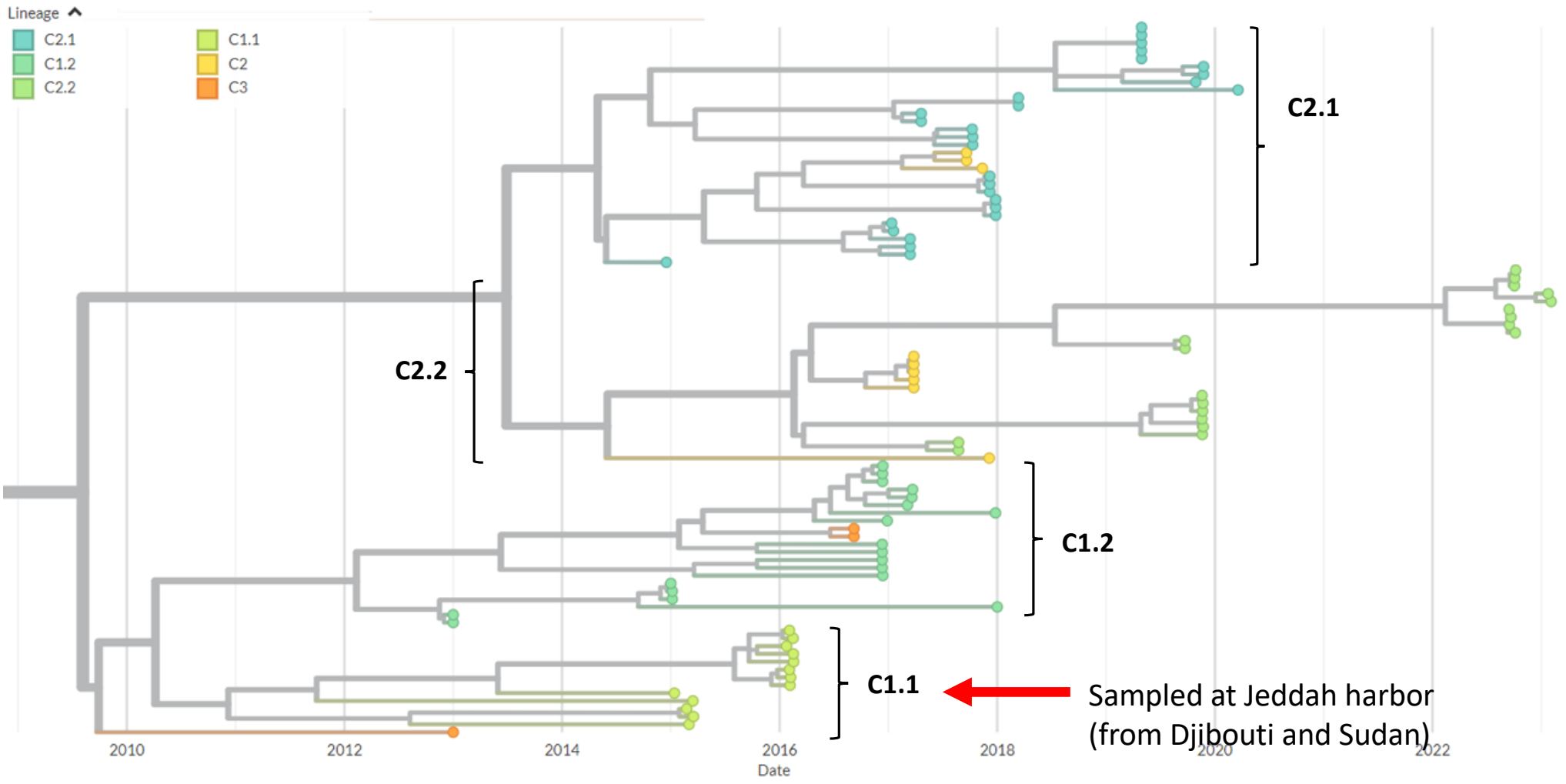
Growth in human lung cells

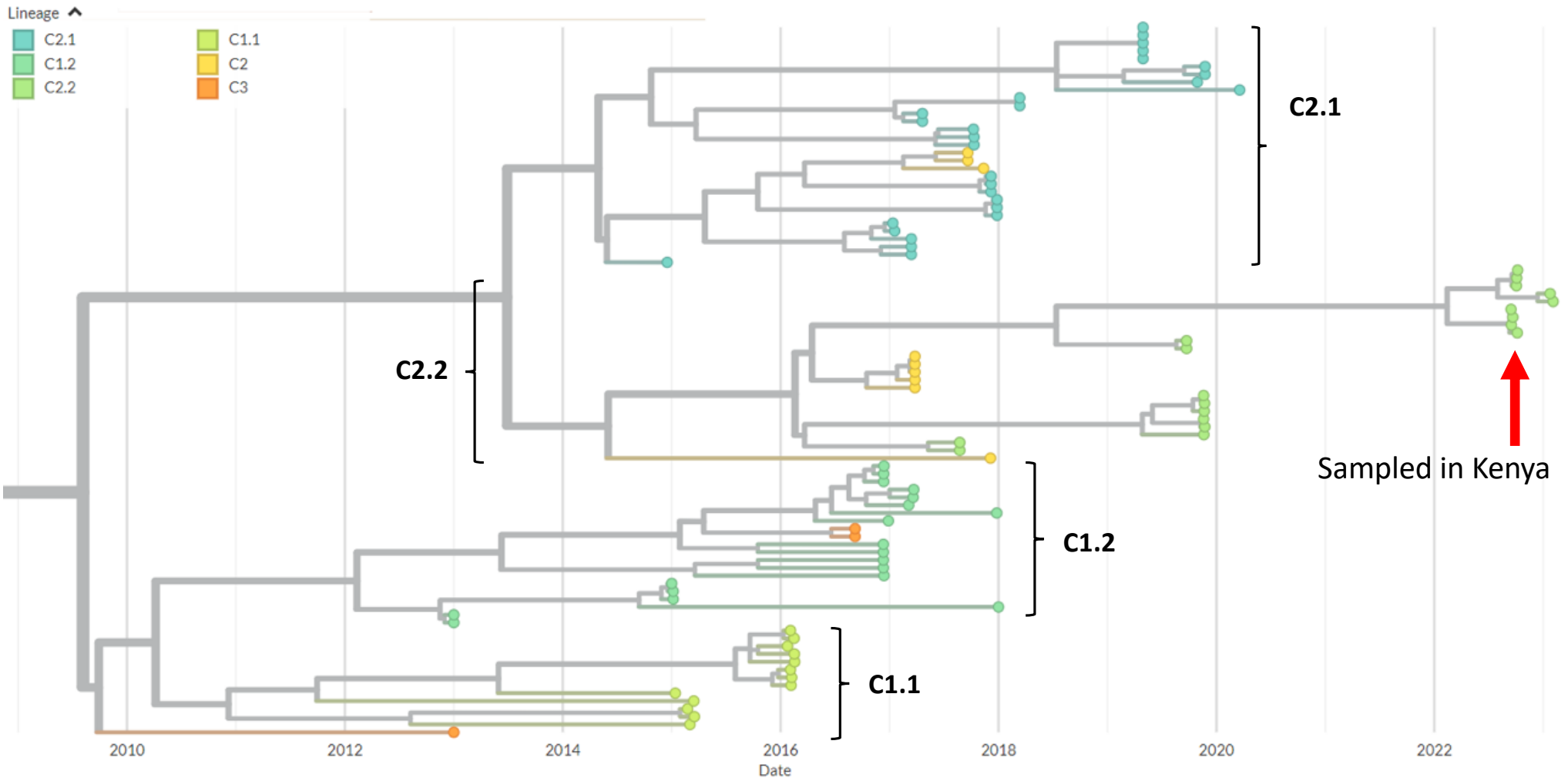


Calu-3
H1299

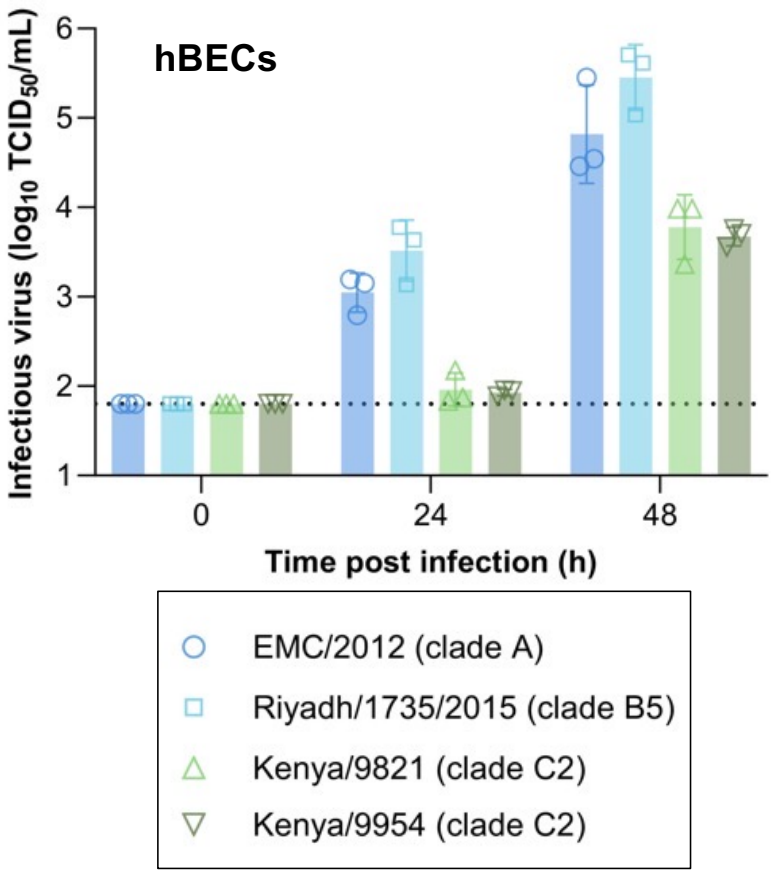


Friedmann, unpublished

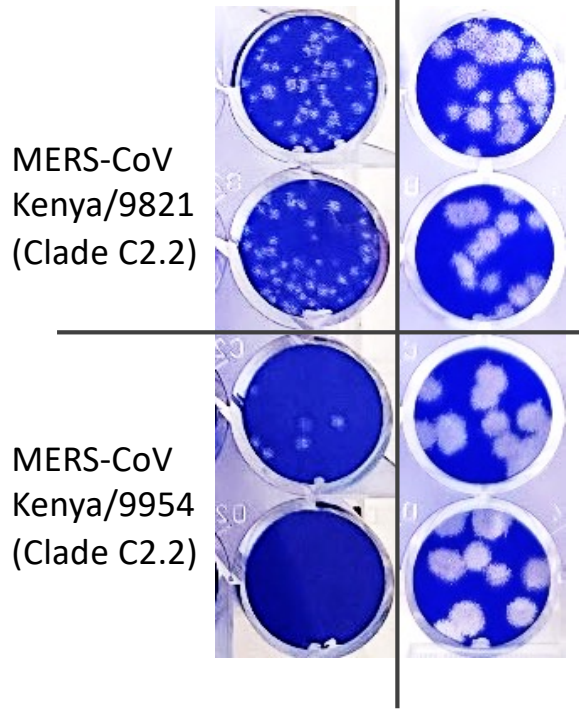

Lineage
C2.1
C1.2
C2.2
C1.1
C2
C3
C2.1
C2.2
C1.2
C1.1
Sampled at Jeddah harbor
(from Djibouti and Sudan)
2010
2012
2014
2016
2018
2020
2022
Date


Lineage
C2.1
C1.2
C2.2
C1.1
C2
C3
C2.1
C2.2
C1.2
C1.1
Sampled in Kenya
2010
2012
2014
2016
2018
2020
2022
Date

Reduced growth in differentiated human bronchial epithelial cultures

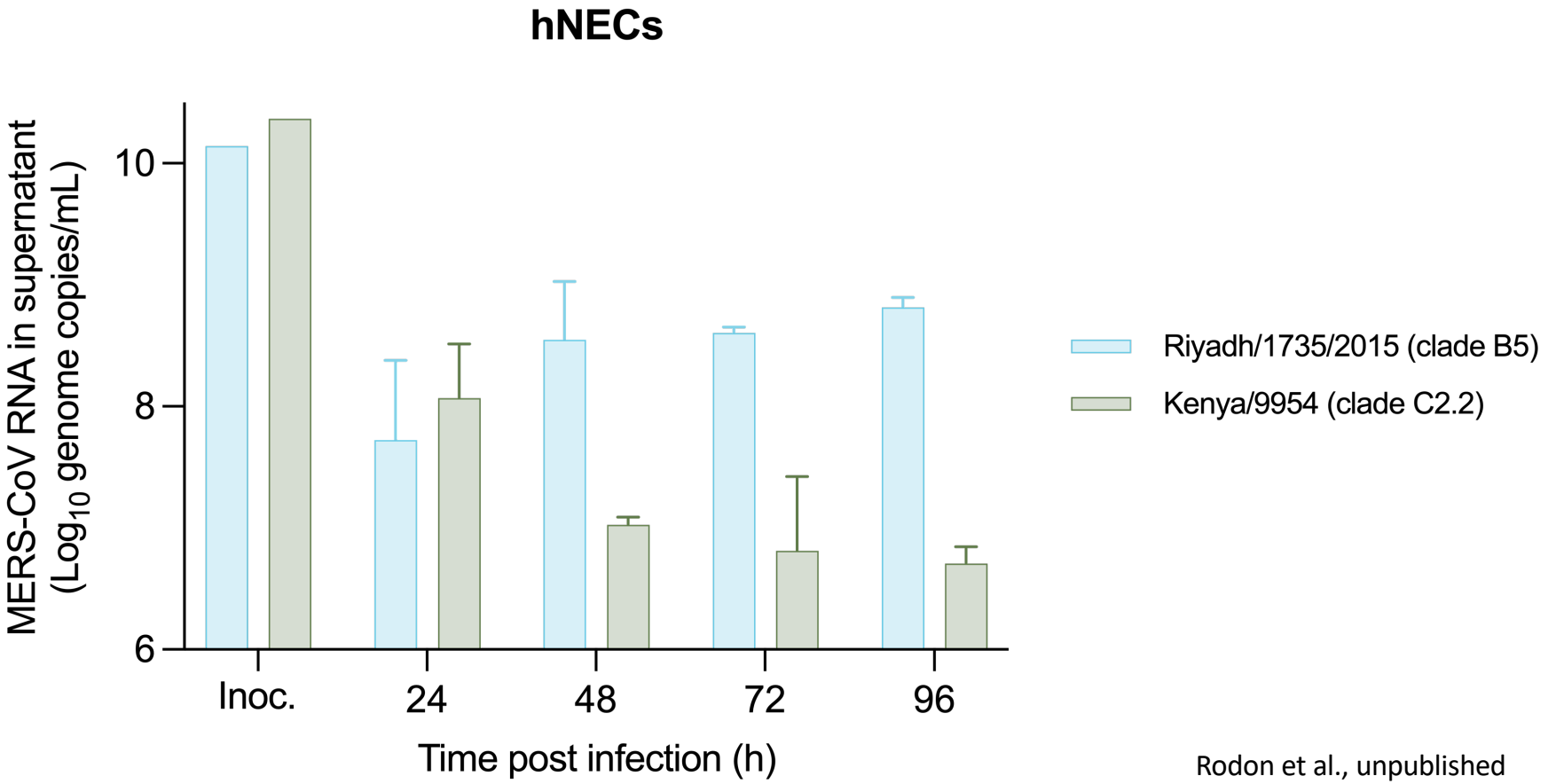


hBEC supernatant
Plaque assay in Vero E6-T, read out at 72hpi



Rodon, unpublished

Impaired growth in differentiated human nasal epithelial cultures



Rodon et al., unpublished

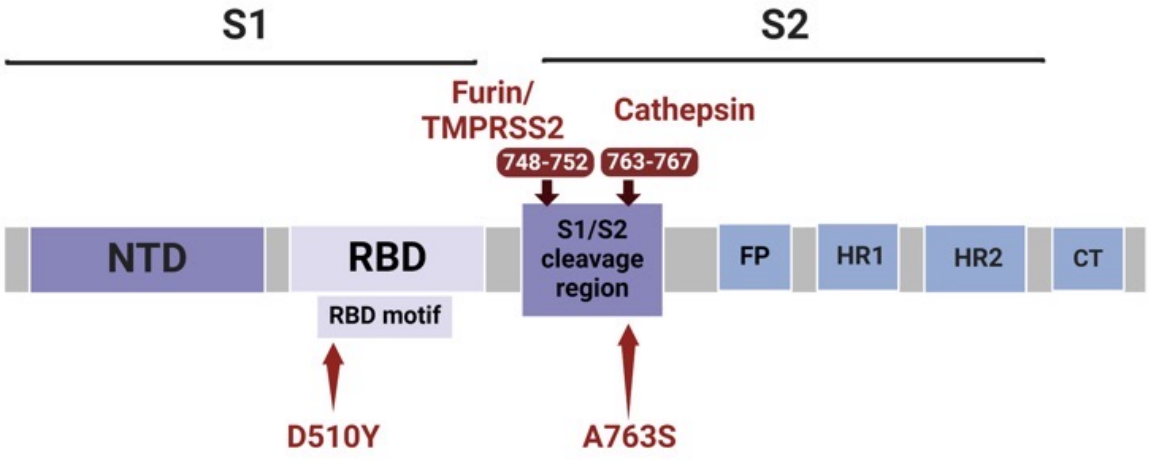
Genome features of MERS-CoV /Kenya 2022-24 strains, clade 2.2

Spike changes (vs EMC strain)

N-terminus:
V26A
D158Y
H194Y

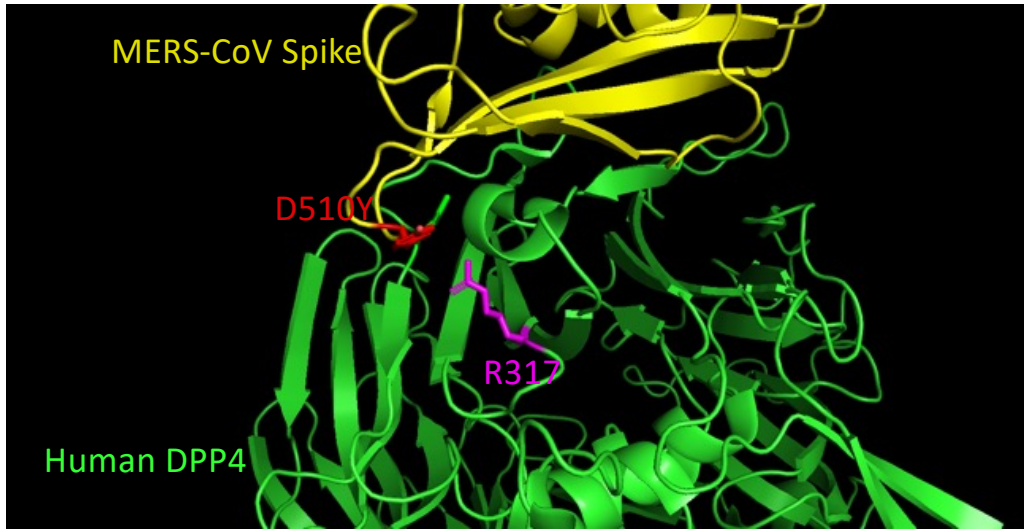
Receptor binding domain:
S390F
L495P
D510Y

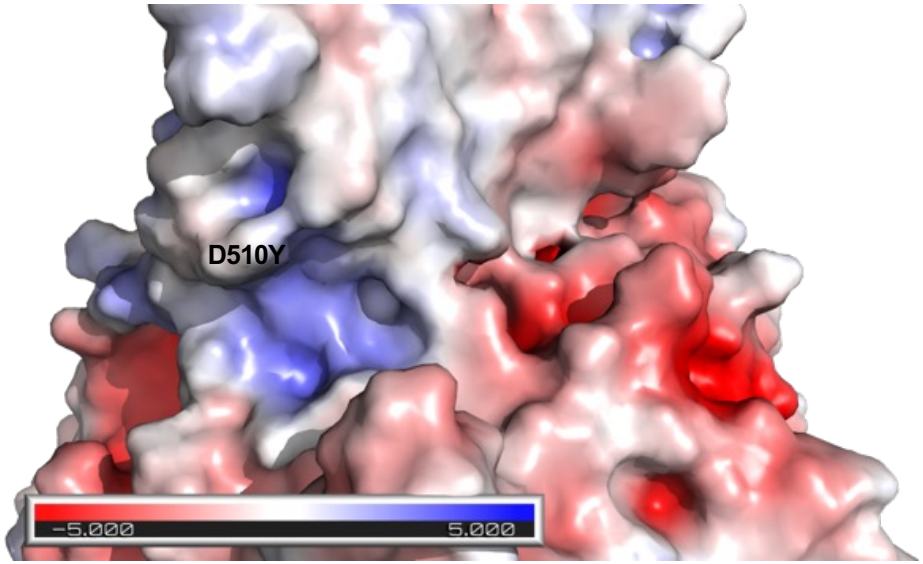
S1/S2:
A597V
R626P
A763S



Rodon, unpublished

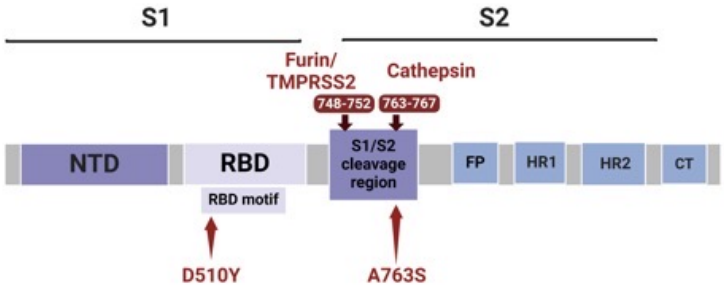
Electrostatical evaluation of Kenya D510Y spike mutant





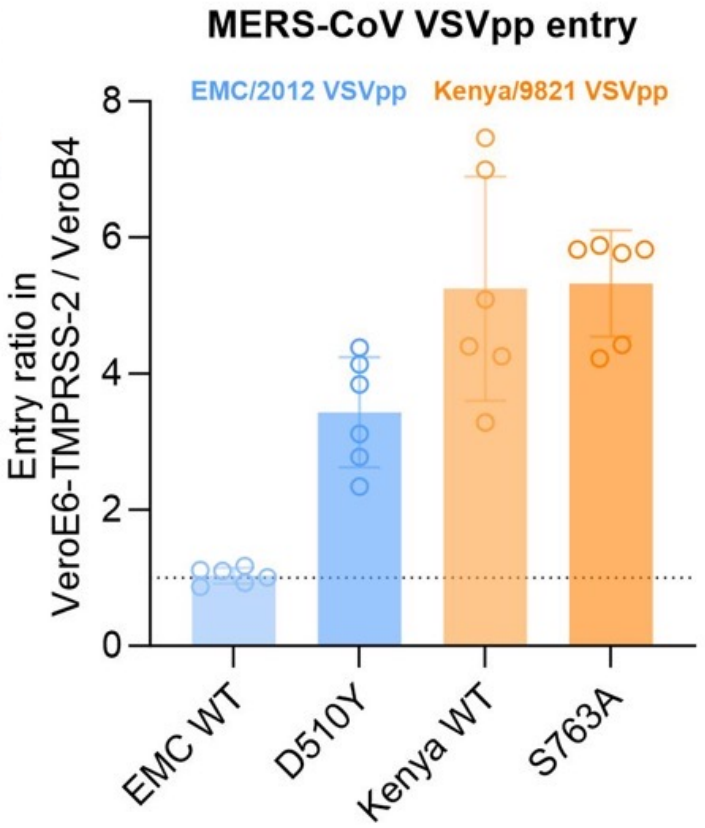
→ Wild-type **510D** residue creates an area of **negative charge** potential around the 507-511 binding loop
→ **D510Y (all new Kenyan sequences) significantly reduces negative charge potential** around the 507-511 binding loop
→ **Would be unlikely to build hydrogen bond with DPP4 R317**

TMPRSS-2-dependent entry of VSVpp-MERS-S



D510Y may increase TMPRSS2 dependence

Reversion of cathepsin-site mutation has no effect

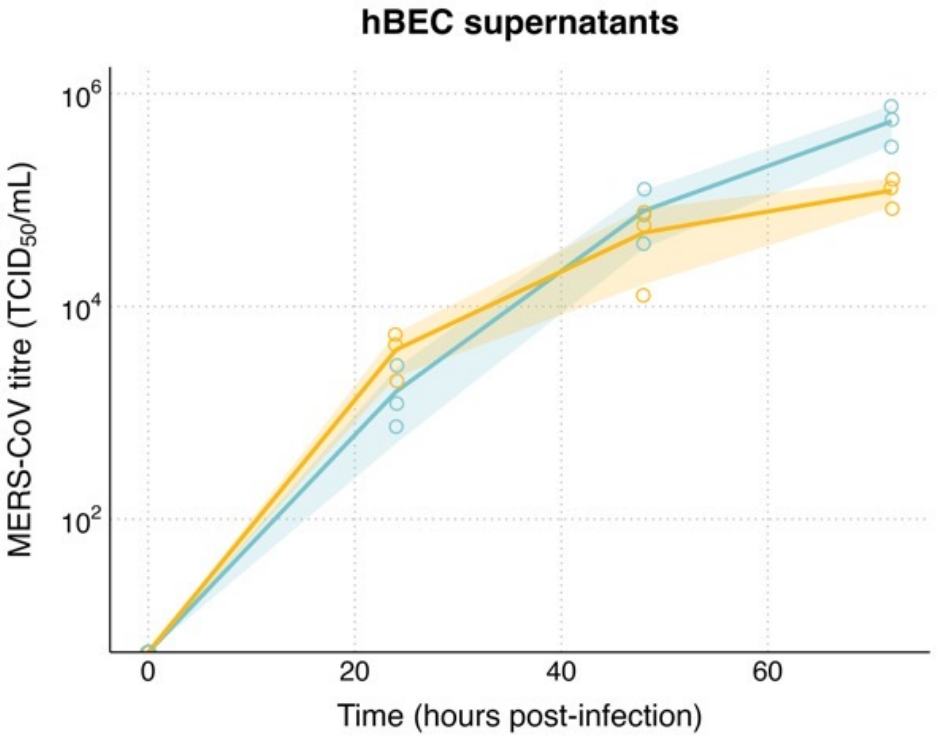


TMPRSS-2 dependence

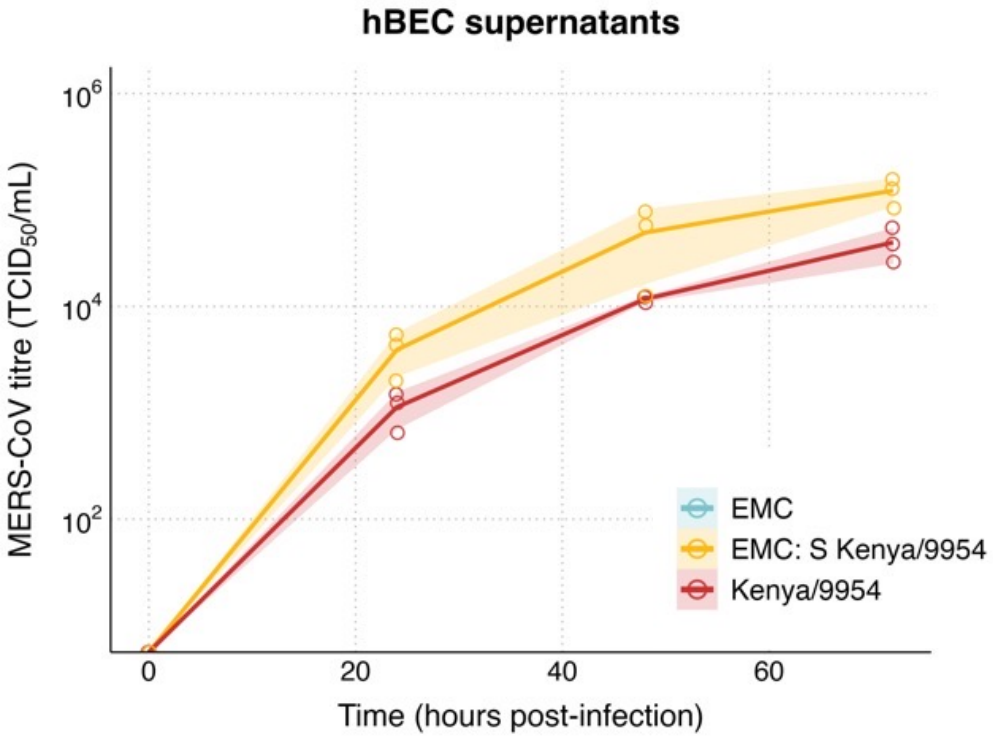
Rodon, unpublished

MERS-CoV Spike and backbone contribute to phenotypic differences

Spike fitness

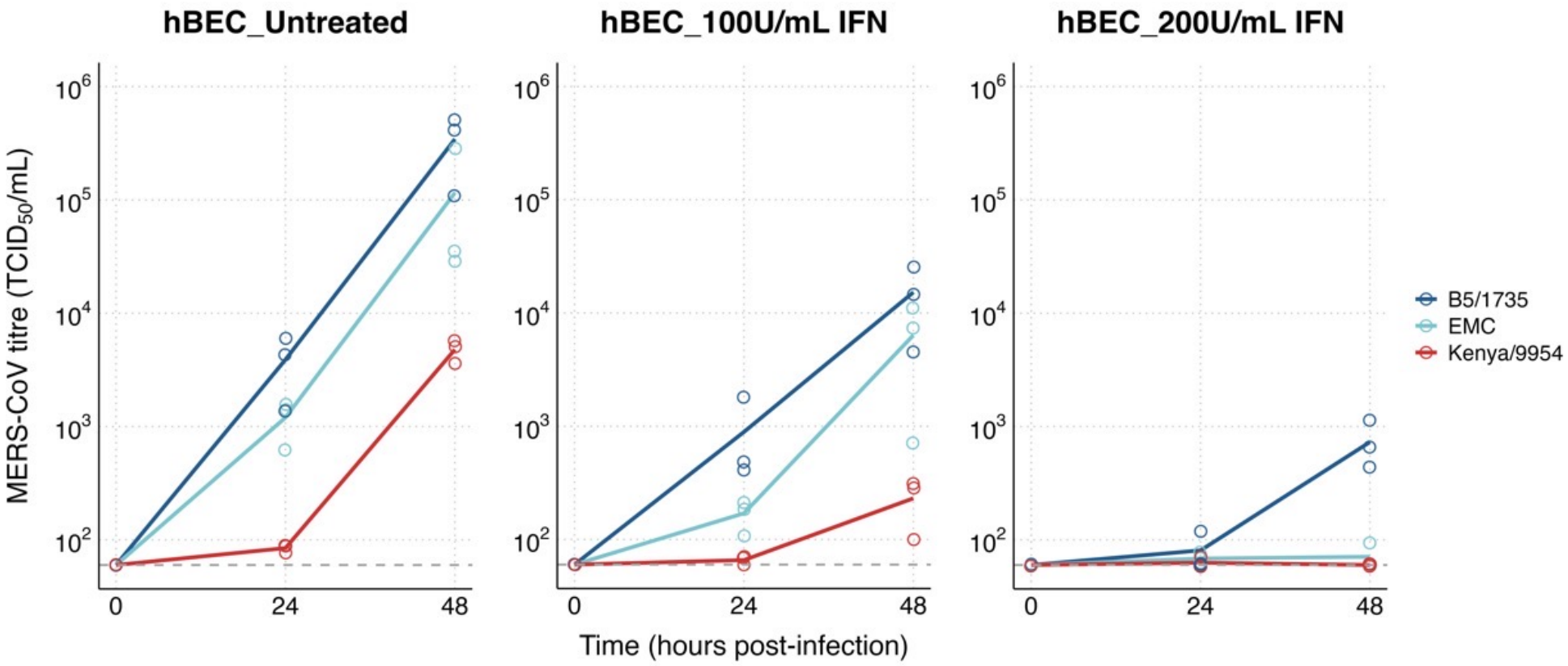


Backbone fitness



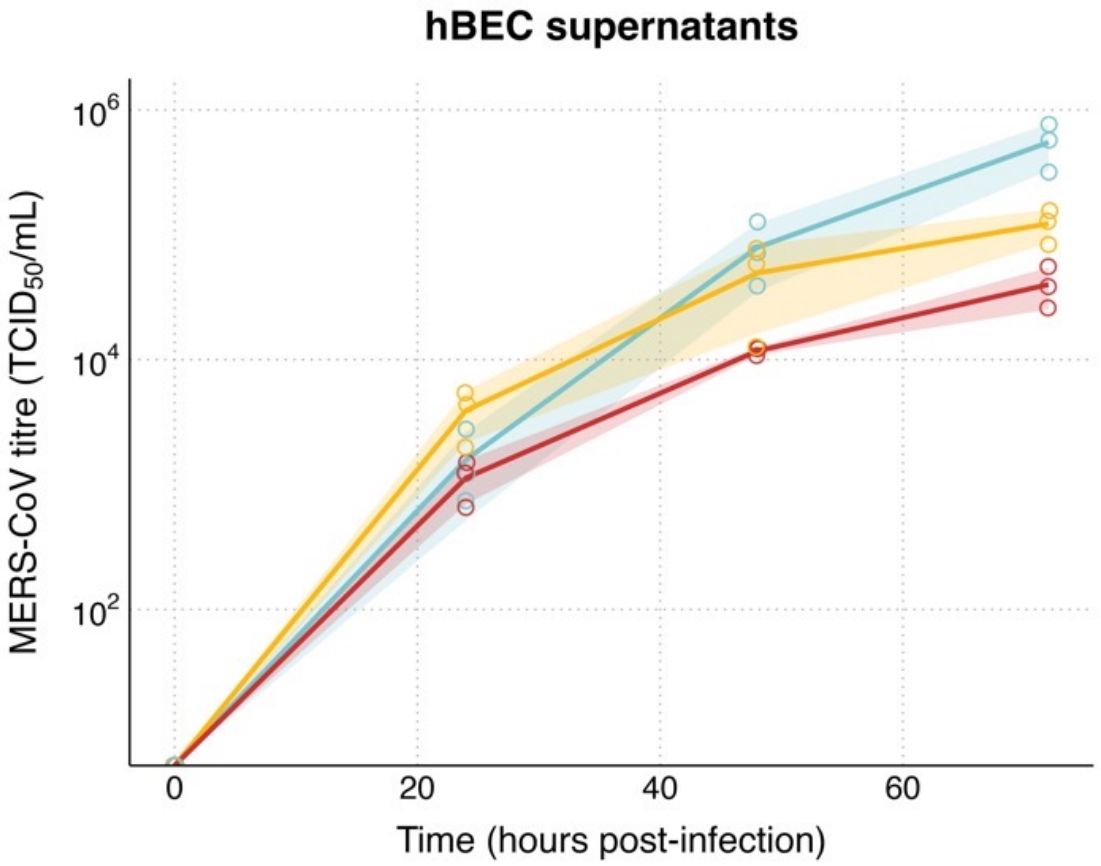
Rodon*, Ogoti*, …. Drosten*, Müller*, unpublished

Higher IFN sensitivity of Kenyan C2.2 strains in hBECs



Rodon*, Ogoti*, …. Drosten*, Müller*, unpublished

Mapping genotype-phenotype fitness through chimeric viruses

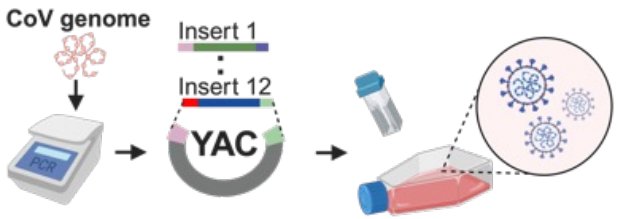




Ref. variant (EMC)

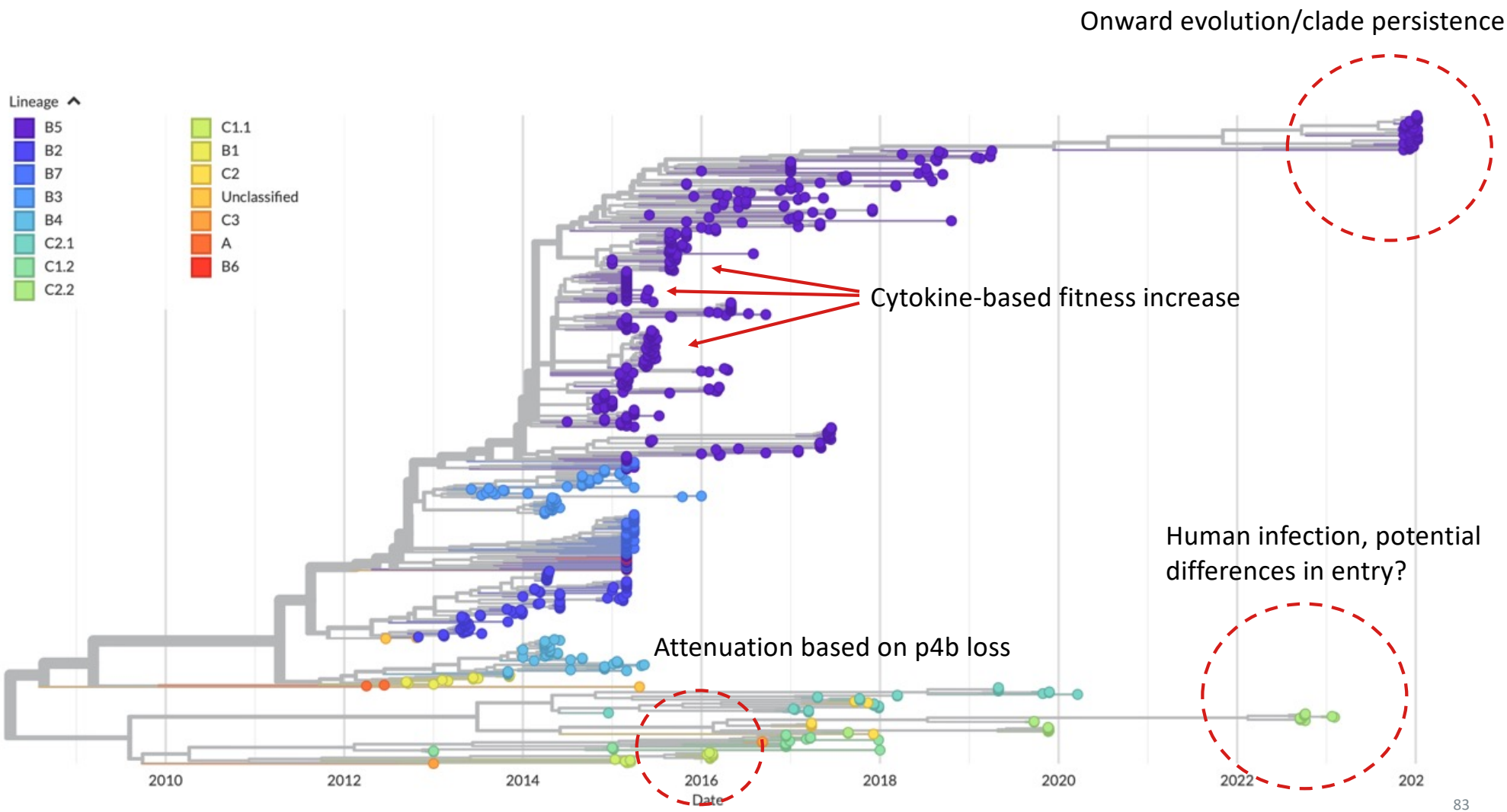
Chimeric virus (EMC: Spike Kenya/9954)

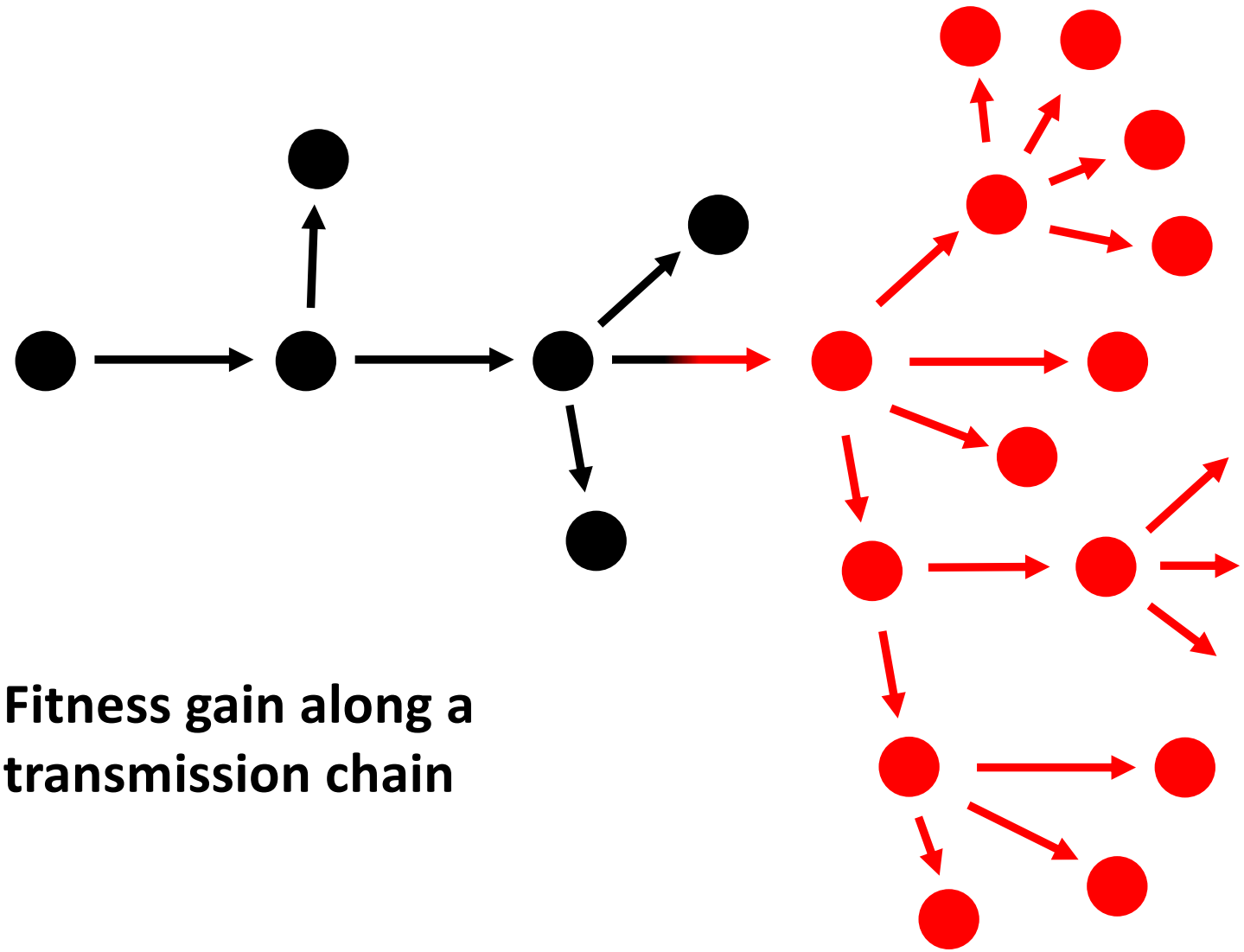
New variant (Kenya/9954)



Created with BioRender

Rodon*, Ogoti*, …. Drosten*, Müller*, unpublished


Onward evolution/clade persistence
Lineage
B5
B2
B7
B3
B4
C2.1
C1.2
C2.2
C1.1
B1
C2
Unclassified
C3
A
B6
Cytokine-based fitness increase
Human infection, potential differences in entry?
Attenuation based on p4b loss
2010
2012
2014
2016
2018
2020
2022
202
Date



Fitness gain along a transmission chain

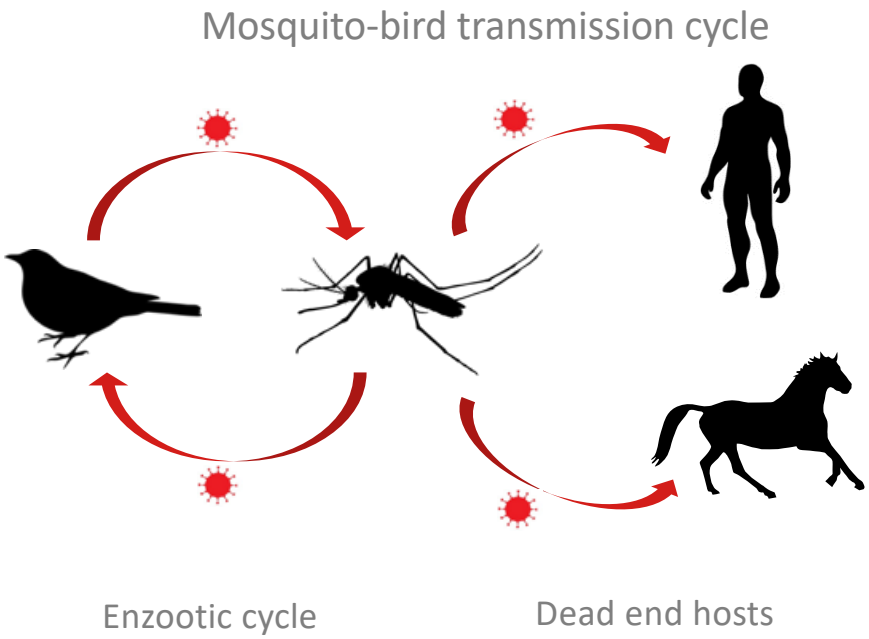
List of candidate vaccines developed against MERS-CoV

Platform	Type of candidate vaccine	Developer	Coronavirus target	Current stage of clinical evaluation/regulatory status- Coronavirus candidate	Non-coronavirus candidates	Current stage of clinical evaluation/regulatory status - other products
DNA	DNA plasmid vaccine that encodes the MERS CoV spike (S) glycoprotein Electroporation device [12-15]	Inovio Pharmaceuticals	MERS	Phase I	Lassa, Nipah (CEPI) HIV Filovirus HPV Cancer indications Zika Hepatitis B	Phase III
Non-Replicating Viral Vector	MVA-MERS-S [1-5]	IDT Biologika GmbH	MERS	Phase I	CHIK, Ebola, Lassa, Marburg, Zika, influenza	Licensed Product
Non-Replicating Viral Vector	ChAdOx1 MERS [6-9]	University of Oxford	MERS	Phase I	Malaria, Influenza, CHIK, RABV, TB, RVFV	Phase I

….and 46 pre-clinical candidates, as listed by WHO

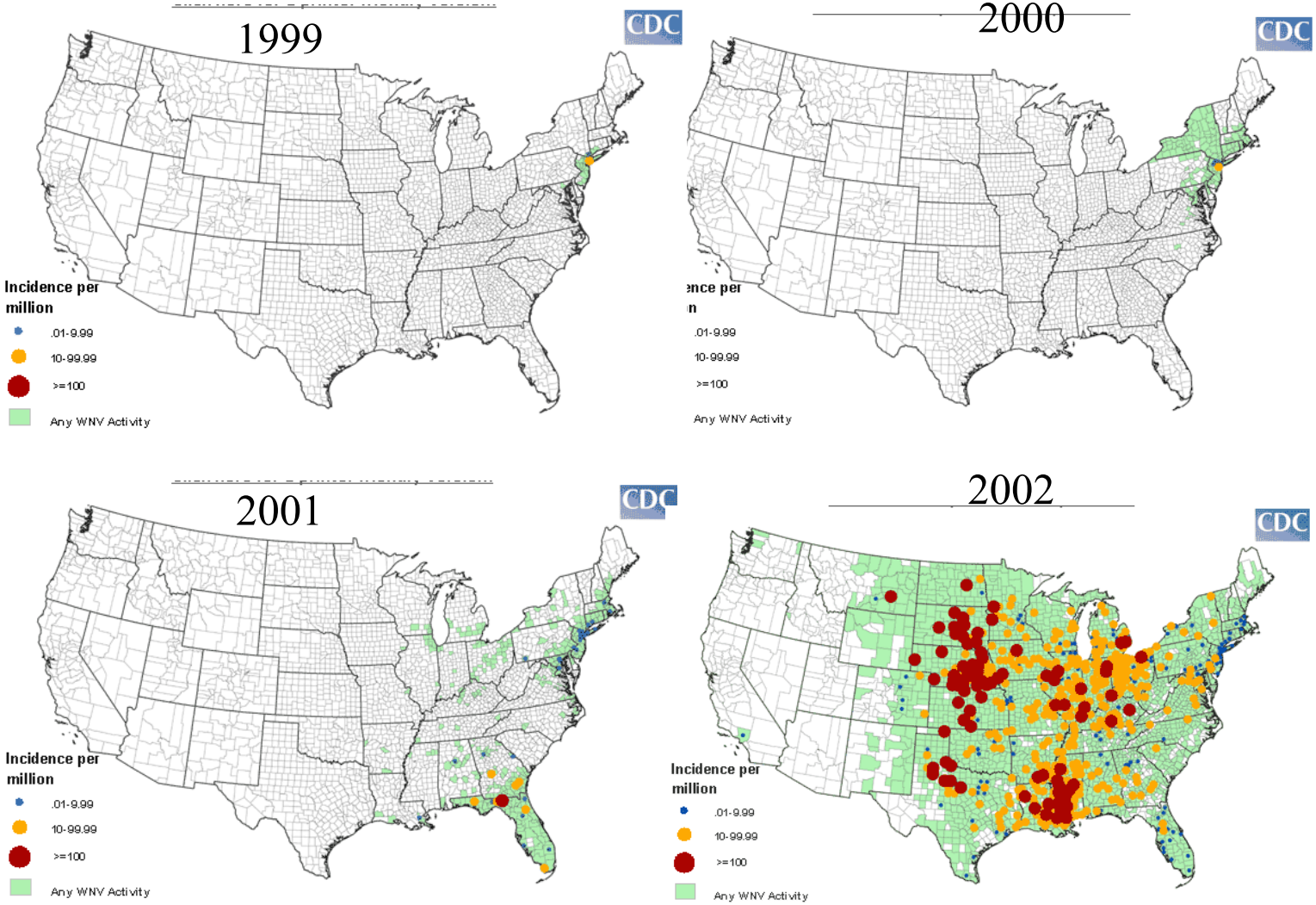
West Nile Virus

West Nile virus (WNV) – Transmission cycle and symptoms



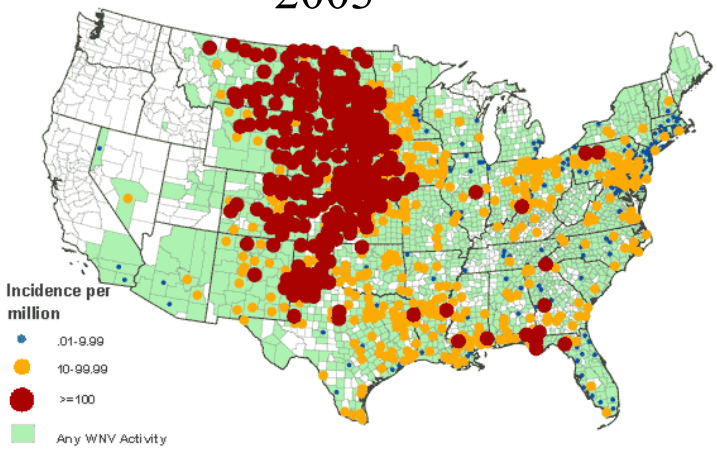
- 75% asymptomatic
- 25% West Nile Fever (WNF)
  - Symptoms: fever, headache, arthralgia, GI symptoms, rash
- 0.7% West Nile Neuroinvasive Disease (WNND)
  - Symptoms: viral meningoencephalitis. CFR 10%.
  - Elderly and immunosuppressed people at risk.

Incidence of neuroinvasive cases, USA, 1999 - 2006

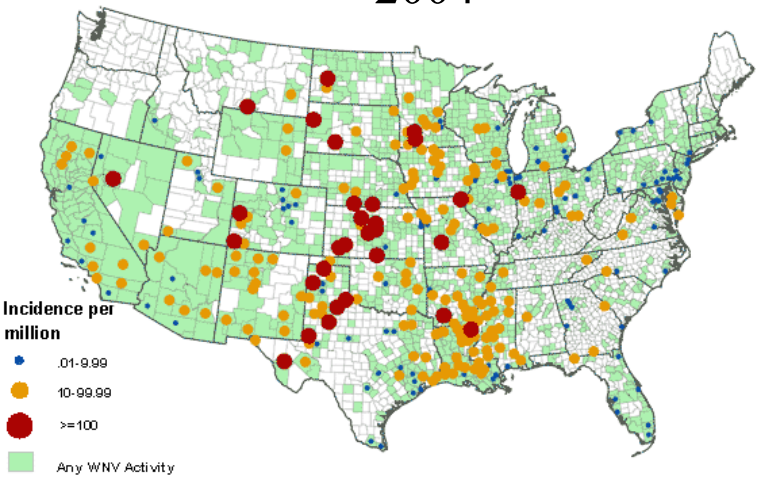


Incidence of neuroinvasive cases, USA, 1999 - 2006

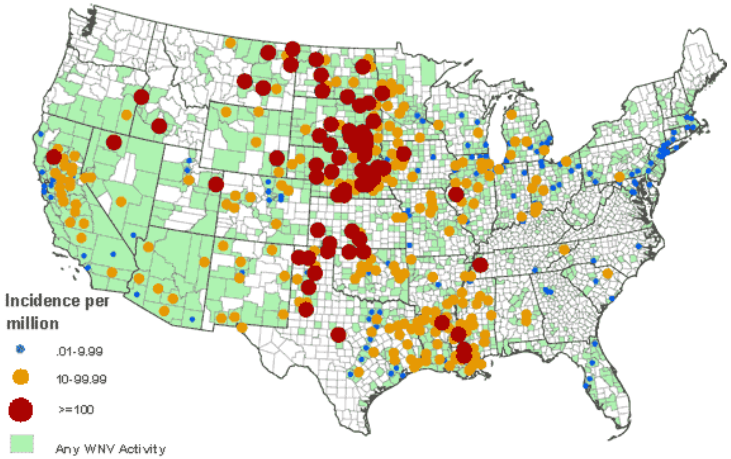
2003



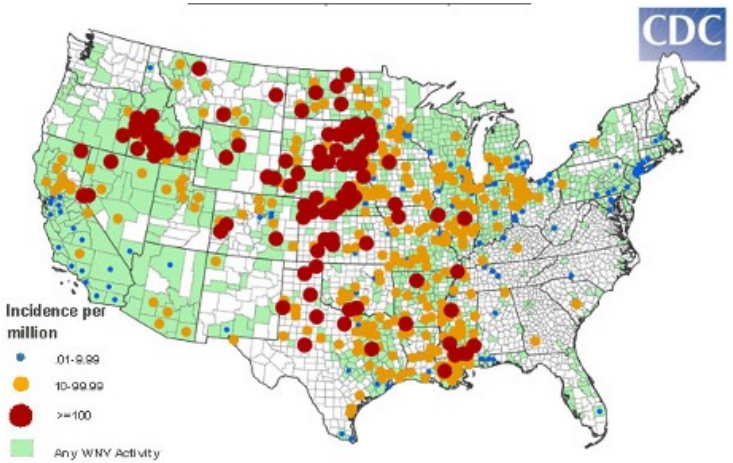
2004



2005



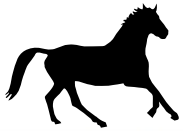
2006



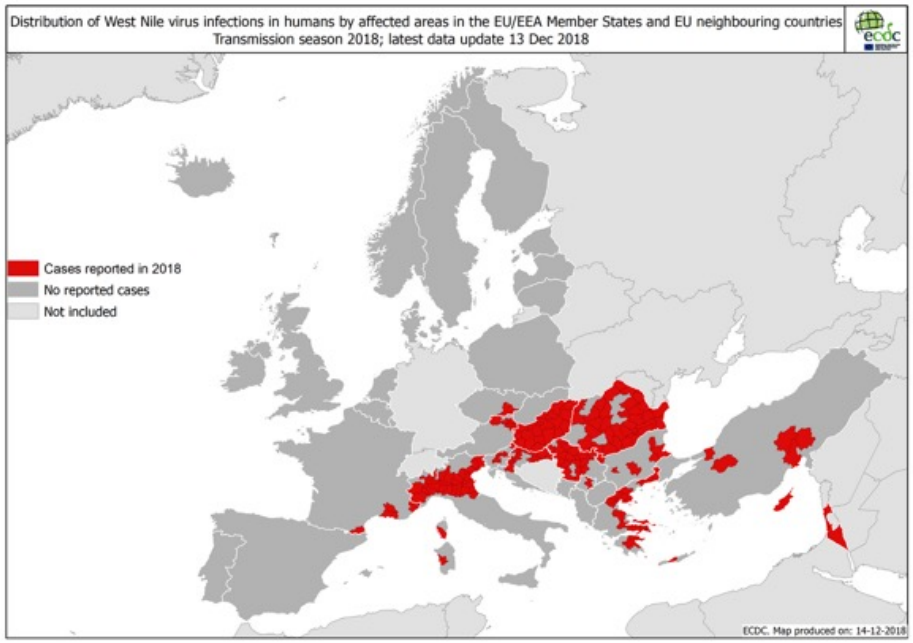
Pan-European outbreak in 2018

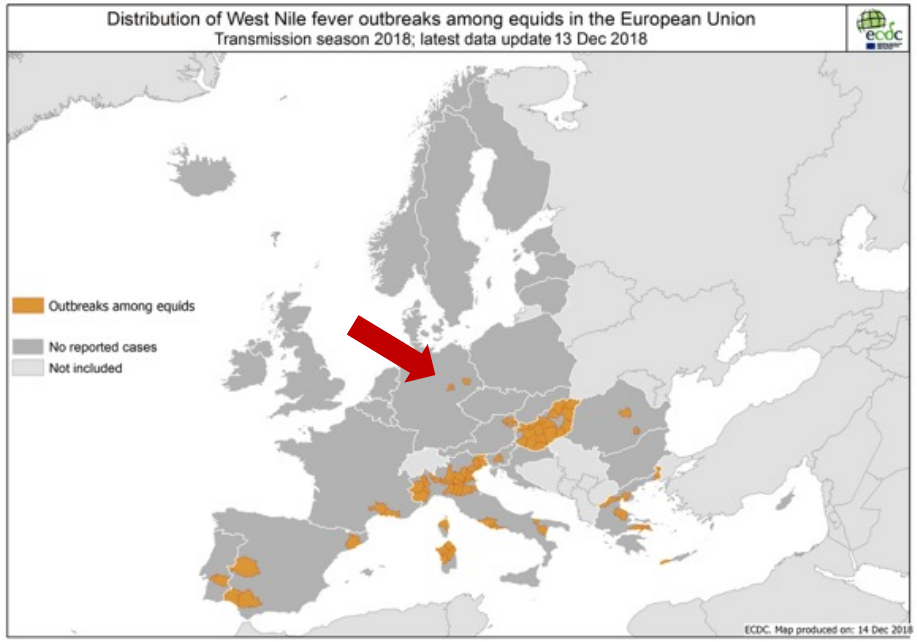


1503 cases



285 cases





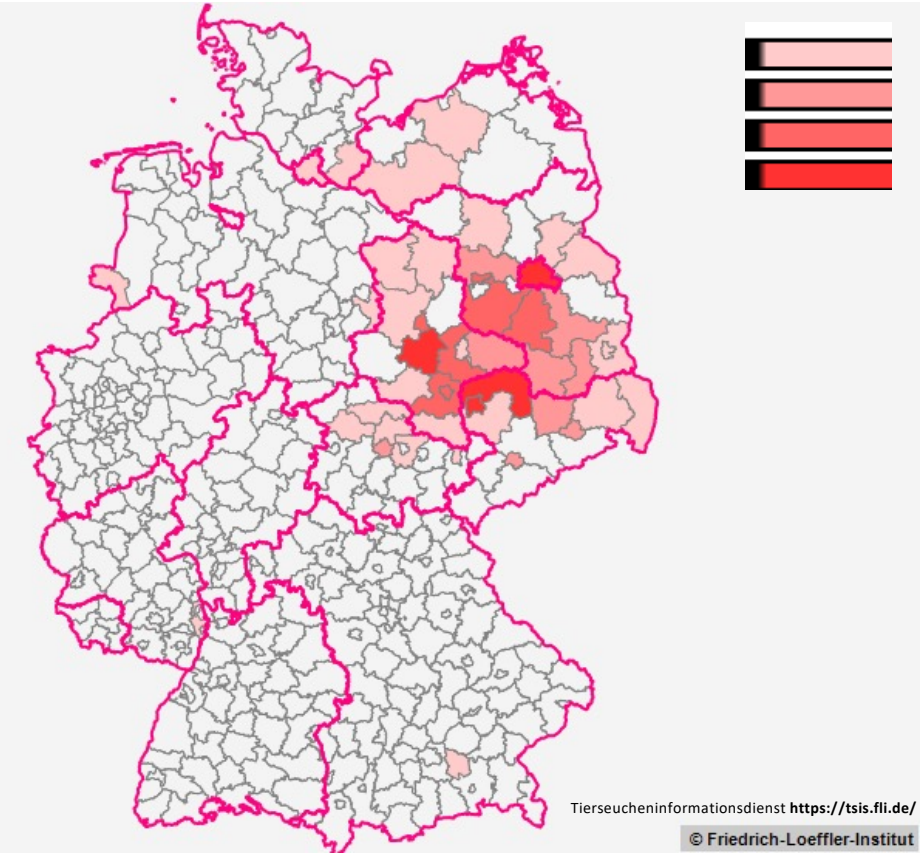
First WNV detection in Germany (2 horses and 12 birds).

West Nile virus (WNV) is endemic in Germany

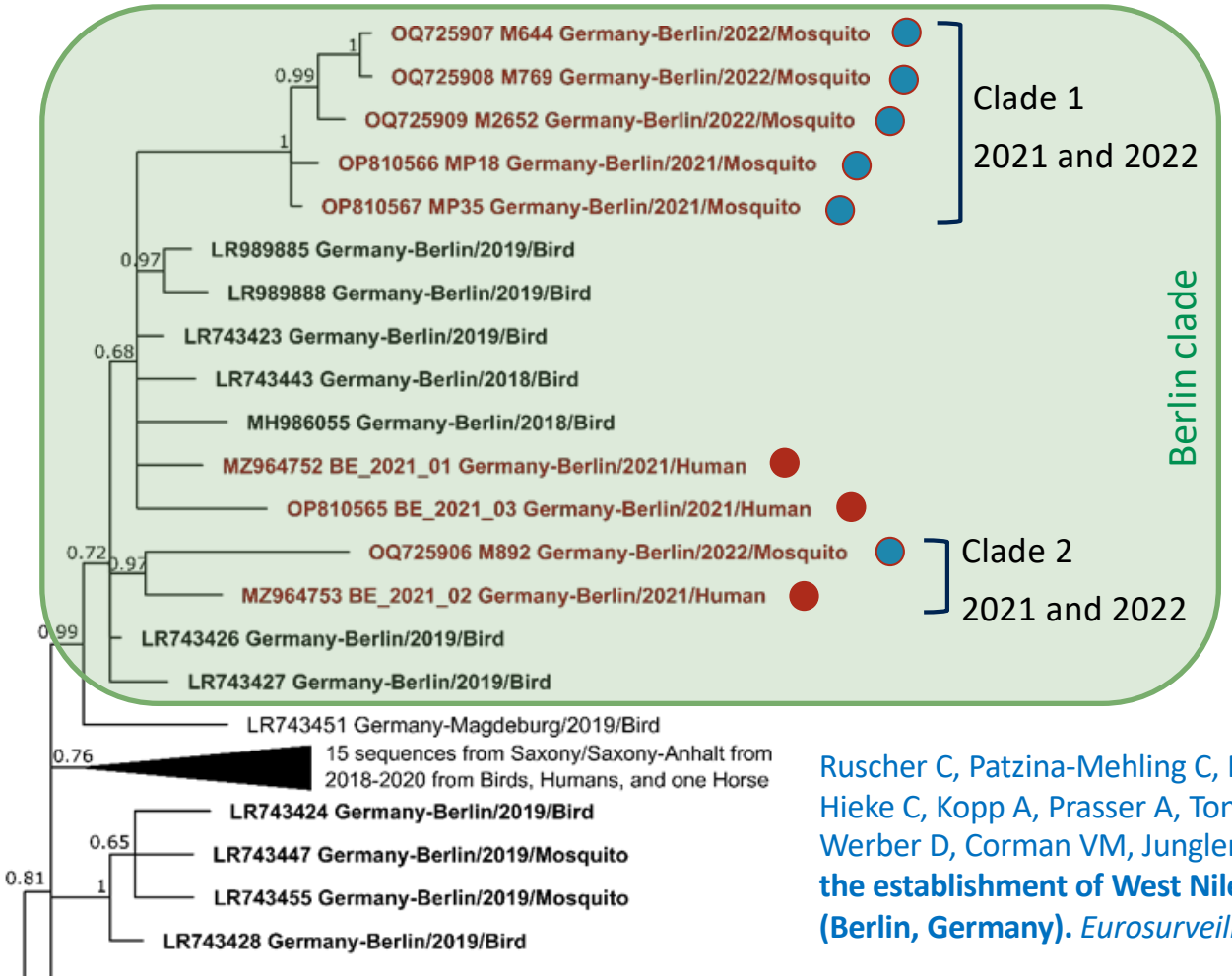
Detections in vertebrate hosts, Germany

2018	2019	2020	2021	2022	2023
	5	23	5	19	4
2	36	22	17	16	2
12	76	63	25	49	17

WNV cases in birds and horses, 2018-2023

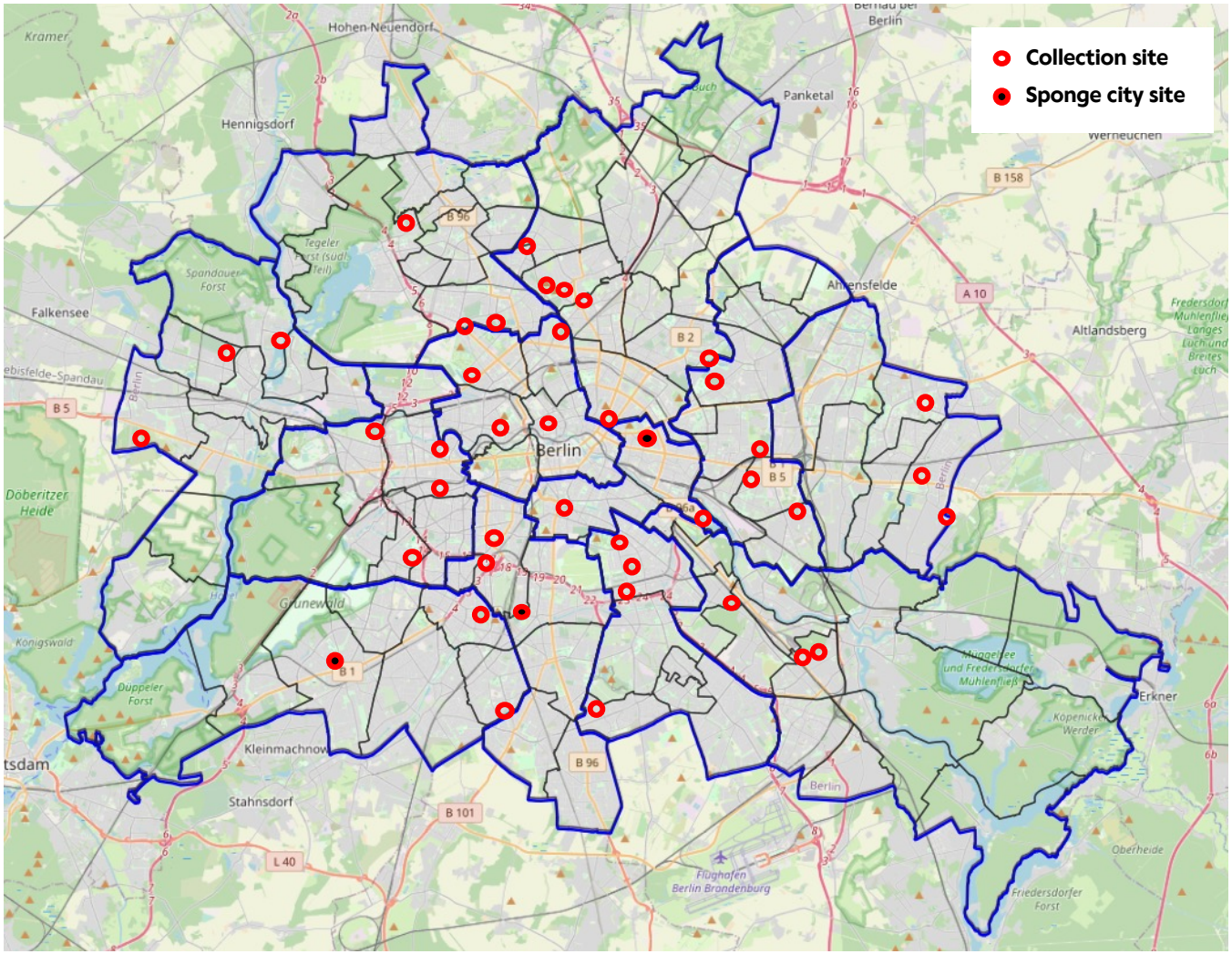


West Nile virus has established an endemic maintenance cycle in Berlin



Ruscher C, Patzina-Mehling C, Melchert J, Graff SL, McFarland SE, Hieke C, Kopp A, Prasser A, Tonn T, Schmidt M, Isner C, Drosten C, Werber D, Corman VM, Junglen S. **Ecological and clinical evidence of the establishment of West Nile virus in a large urban area in Europe (Berlin, Germany).** *Eurosurveillance* 2023 Nov;28(48):2300258.

Sampling of mosquitoes across Berlin, 2023



43 sites:

3 – 6 / district

Cemeteries, garden plots, gardens

CHARITÉ

DURABLE
Research Network for Epidemics

West Nile Virus Vaccine Candidates Tested in Clinical Trials.*

Vaccine Type, Name, and Trial	Age of Participants (yr)	No. of Participants Enrolled	No. and Timing of Doses	Immunogenicity	Serious Adverse Events Attributed to Vaccine	ClinicalTrials.gov Number
Live attenuated chimeric						
WN/DEN4-3′Δ30 (NIAID, NIH)						
Phase 1 trial, completed in 2005	18–50	56	1 Dose	55–75% Seroconversion (increase in $PRNT_{60}$ by a factor of ≥4 at day 28 or 42)	None	NCT00094718
Phase 1 trial, completed in 2009	18–50	26	2 Doses, 6 mo apart	89% Seroconversion after second dose (increase in $PRNT_{50}$ by a factor of ≥4 at day 28 or 42)	None	NCT00537147
Phase 1 trial, completed in 2016	50–65	28	2 Doses, 6 mo apart	95% Seroconversion after first dose by day 90 ($PRNT_{50}$ ≥1:10)	None	NCT02186626
ChimeriVax-WN02, YFV 17D backbone (Sanofi Pasteur)						
Phase 2 trial, completed in 2009						
Part 1	18–40	112	1 Dose	>96% Seroconversion (increase in $PRNT_{50}$ by a factor of ≥4 at day 28)	None	NCT00442169
Part 2	≥41	96	1 Dose	Approximately 96% seroconversion (increase in $PRNT_{50}$ by a factor of ≥4 at day 28); GMT and viremia tended to be higher among persons ≥65 yr of age	None	NCT00442169
Phase 2 trial, completed in 2009	≥50	479	1 Dose	92–95% Seroconversion (increase in $PRNT_{50}$ by a factor of ≥4 at day 28)	None	NCT00746798
DNA						
VRC-WNVDNA017-00-VP, first generation (NIAID, NIH, DVBD, CDC)						
Phase 1 trial, completed in 2008	18–50	15	3 Doses, 4 wk apart	100% Had neutralizing antibodies detected ($PRNT_{50}$) at 4 wk and 24 wk after third dose; titers were similar to those elicited in horses 3 wk after 1 dose of WNV DNA vaccine	None	NCT00106769
VRC-WNVDNA020-00-VP, second generation (NIAID, NIH)						
Phase 1 trial, completed in 2007	18–65	30	3 Doses, 4 wk apart	>96% Had neutralizing antibodies detected (EC_{50}) at 4 wk after third dose	None	NCT00300417
Recombinant subunit						
WN-80E (Hawaii Biotech)						
Phase 1 trial, completed in 2009	18–45	25	3 Doses, 4 wk apart	Unpublished	Unpublished	NCT00707642
Inactivated whole virus						
HydroVax-001, hydrogen peroxide inactivated (Najit Technologies)						
Phase 1 trial, completed in 2016	18–49	51	2 Doses (1 μg or 4 μg), 4 wk apart	31–50% Seroconversion at 15 days after second dose ($PRNT_{50}$ ≥20 with or without complement enhancement)	None	NCT02337868
Formalin inactivated (Nanotherapeutics)						
Phase 1–2 trial, completion date unknown	≥18	320	2 Doses, 3 wk apart; booster on day 180	All doses elicited immune response, with peak antibody responses after booster dose (microneutralization)	None	None

* PRNT (plaque reduction neutralization test) titers are expressed as the reciprocal of the last serum dilution showing the given reduction in plaque counts (50% or 60%). For the WN-80E vaccine, the patent number is US20120141520 A1. CDC denotes Centers for Disease Control and Prevention, DVBD Division of Vector-Borne Diseases, EC effective concentration, GMT geometric mean titer, NIAID National Institute of Allergy and Infectious Diseases, NIH National Institutes of Health, WNV West Nile virus, and YFV yellow fever virus.

CHARITÉ

Gould, NEJM 2023

No vaccine against WNV infection in humans

1999-2021, USA: 55 K cases, 27 K neuroinvasive courses, 2.6 K fatalities.

However, most of these cases occurred during the period of initial spread.

Most cases were thus missed when vaccine candidates had passed phase 1/2.

No vaccine against WNV infection in humans

Since the initial spread, there is extreme spatiotemporal variability in incidence. And only 1/150 cases develops neuroinvasive course against which protection would be targeted.

Efficacy would have to be based on recent vaccination, i.e., before the present season. For all inactivated vaccines, this means: two first course doses plus a refresher per season.

It is close to impossible to show protection in this setting.

Toward vaccination in humans

License/authorize vaccines based on surrogate criteria (correlates of protection). Neutralizing antibodies.

Confirm effectiveness post authorization.

Most likely criterion: reduction of notified cases of neuroinvasive disease.

Also: test-negative design in meningoencephalitis cohorts.

Are we missing something?

Influenza A – unclear situation, new tropism in a livestock species, lack of human surveillance and control

MERS – evolving virus in livestock species, signs of increase of fitness

West Nile – not a pandemic scenario; establishment in temperate zones with US precedent, likely susceptibility of local reservoir ecology