University of Zurich[UZH]

Institute of Medical Virology

First Experience of Clinical Application of Metagenomics in Viral Diagnostics

Michael Huber

Challenges in routine virus diagnostics

Viral pathogens

- more than 200 viruses pathogenic for humans
- rare, emerging, new viruses (SARS-CoV-2)
- frequent co-infections

Routine virus diagnostics

- molecular methods
- highly sensitive
- rapid
- cost effective

- pathogen/sequence-specific
- multiple tests necessary

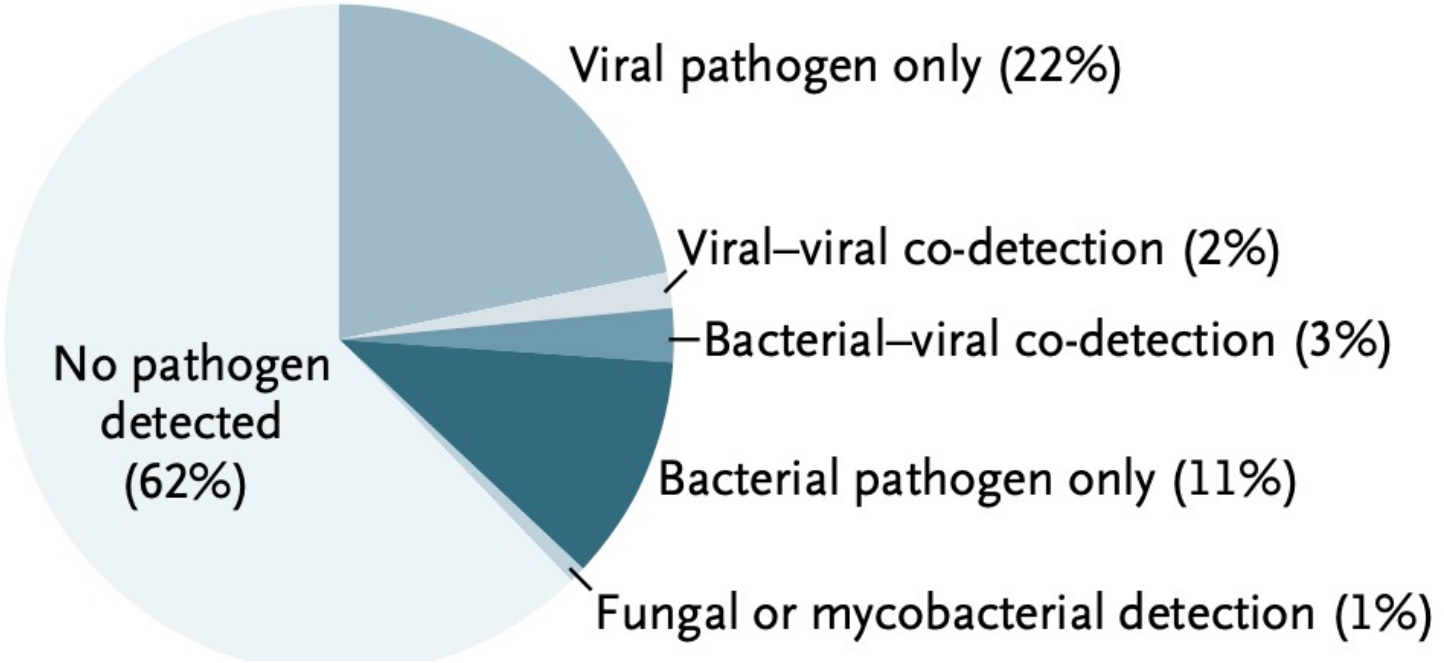
- limited number of tests available

Infections with unknown etiology are frequent

Up to 60% of cases

- Meningitis/encephalitis
- Respiratory infections
- Acute gastroenteritis

Pathogen Detection among U.S. Adults with Community-Acquired Pneumonia Requiring Hospitalization, 2010–2012.



Jain et al, NEJM, 2015

Importance of diagnosis of viral pathogens

- Antiviral treatment is often not available
- Accurate and rapid diagnosis is nevertheless important to
  - reduce unnecessary diagnostic steps
  - install appropriate therapy
  - avoid unnecessary antibiotics treatment
  - reduce healthcare costs
  - enforce or lift isolation
  - limit nosocomial spread

Need for an open diagnostic approach

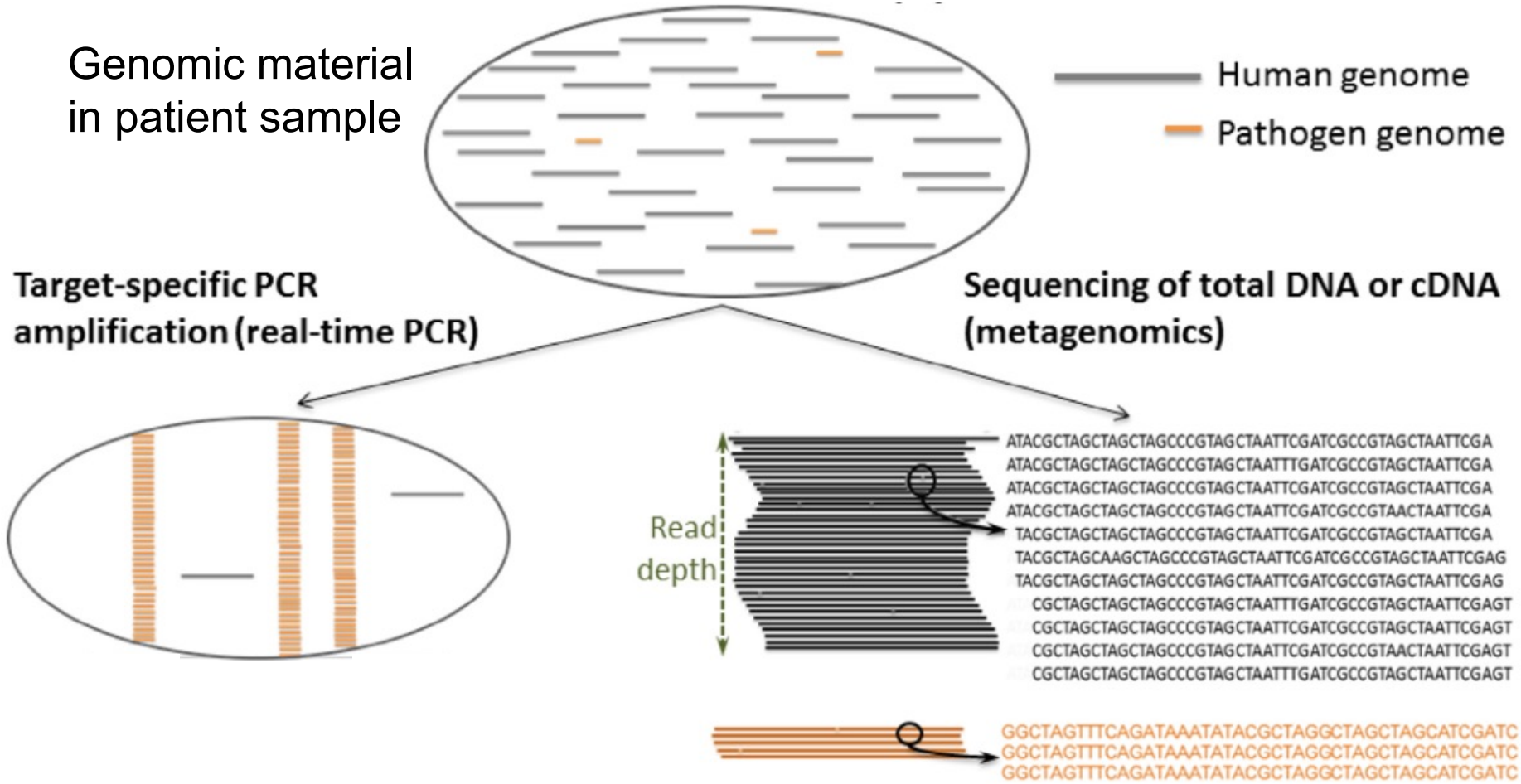
- multiplex PCR panels provide the possibility to screen dozens of pathogens
- development of high-throughput sequencing (NGS) provided unprecedented opportunities
- metagenomic sequencing (mNGS) allows for open, unbiased genomic detection of virtually any viral or other pathogen in clinical samples.

Agenda

- Metagenomic virus sequencing workflow
- A case of rare virus infection resolved by metagenomic sequencing
- Two years of viral metagenomics in a tertiary diagnostics unit
- Viral metagenomics in primary care patients suffering from respiratory infection
- Summary and Outlook

Metagenomic Virus Sequencing Workflow

Unbiased metagenomic sequencing as open diagnostic tool



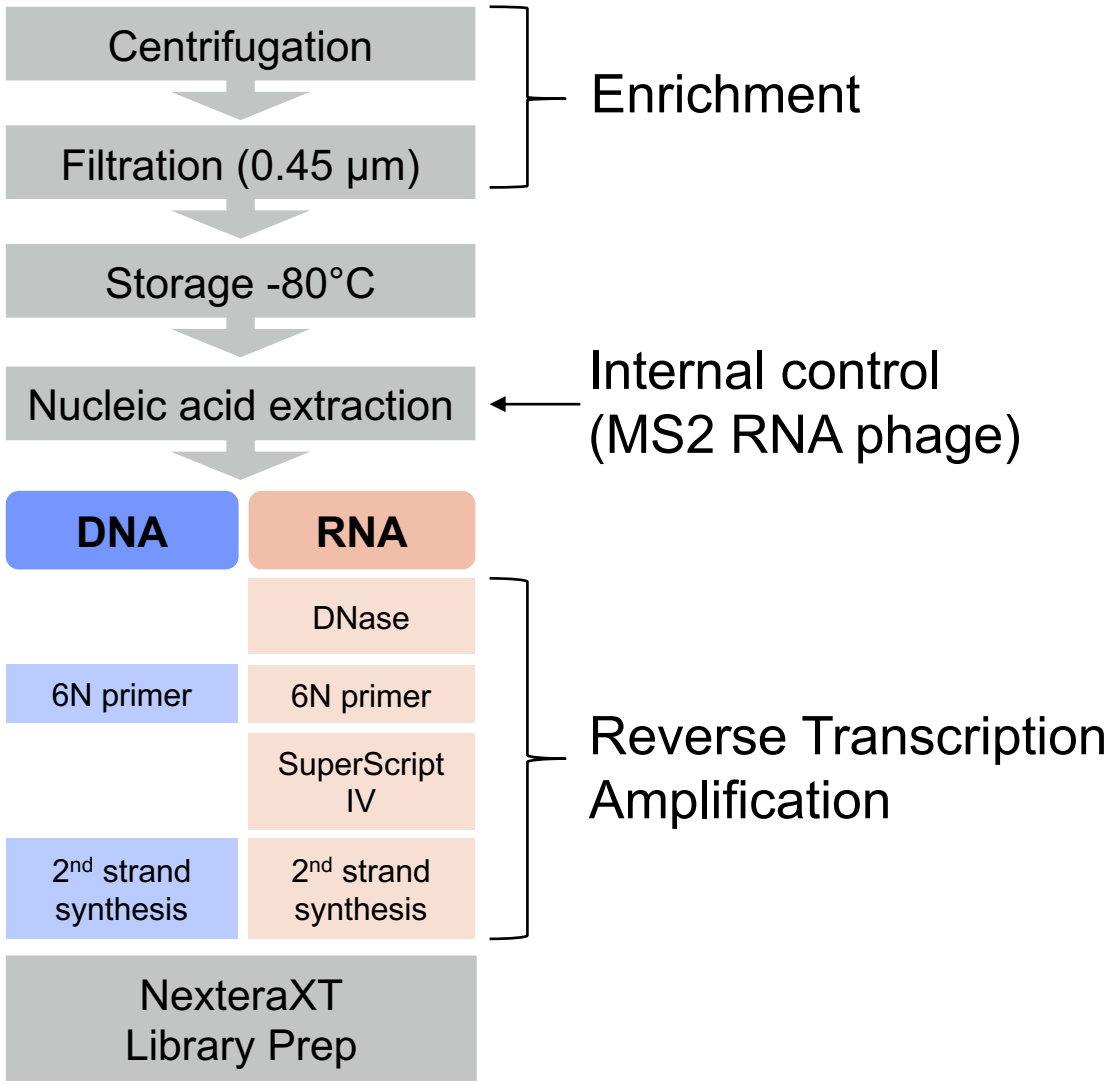
Pros: Fast, inexpensive, sensitive
Cons: Unexpected or novel pathogens not detected; multiple reactions required for multiple pathogens

Pros: Pan-pathogen detection in a single reaction
Cons: Relatively expensive with slower time-to-result than PCR

Brown et al., Journal of Infection, 2018

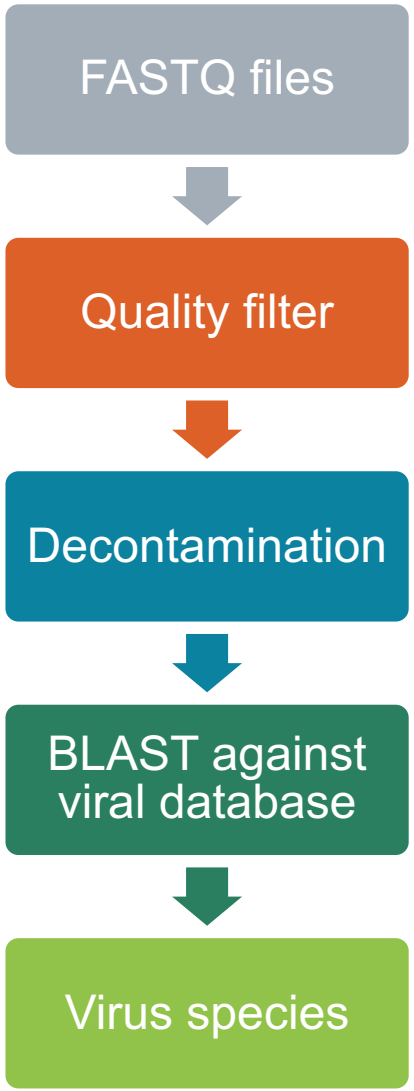
Metagenomic virus sequencing workflow

Clinical Sample (Plasma, CSF, urine, swabs)



- Illumina MiSeq
- 5 samples per run
- 7.5 M reads per sample
- 150 nucleotides read-length
- 16 h run time

Bioinformatic pipeline “VirMet”



>read_1
ATCGTACGTGATCGTACGGGACATACGGCTGGTACGTAGCATCG…
>read_2
CGTGATCGTACGGTGTACCGTCGTACCGAGGACTCGGTGCTGC…

- ✓ quality (PHRED > Q20)
- ✓ length (> 75 nucleotides)
- ✓ high entropy

- – human reads
- – bacterial reads
- – bovine reads

BLAST = Basic Local Alignment Search Tool
in-house data base with >60’000 virus sequences from GenBank

internal control (MS2 phage)
reads assigned to virus species
unaligned reads reported as “unknown”

https://github.com/medvir/VirMet

Metagenomic Virus Sequencing Report

Domain level taxonomy profile

domain	reads	percent	abundance
bacterial	802206	6.394	+++
human	6628827	52.837	++++++++++++++++++++
other	396432	3.160	++
unknown	1093505	8.716	++++
viral	3624762	28.892	++++++++++++

Virus species

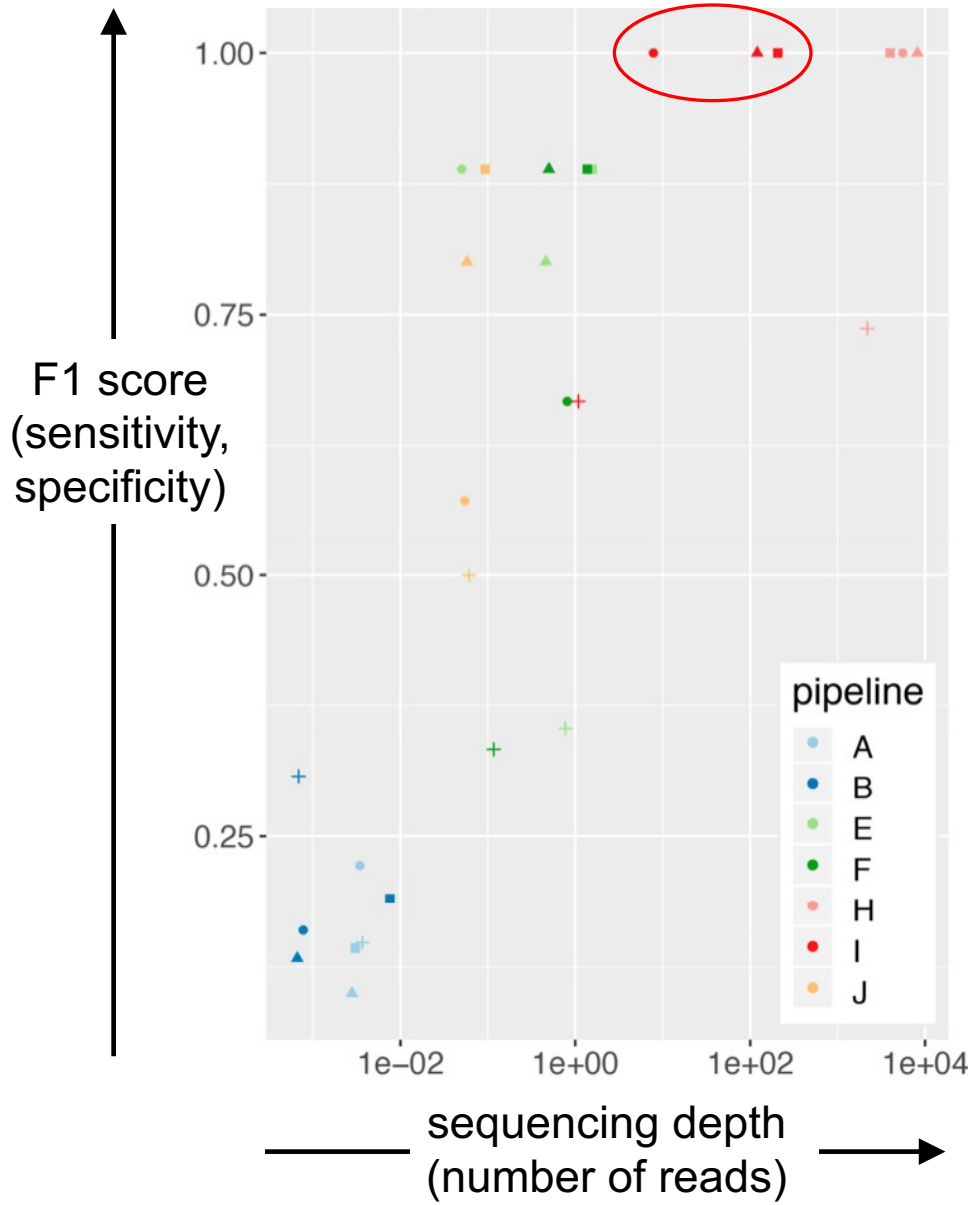
organism	reads	percent	abundance
Coxsackievirus A6	3590604	99.058	++++++++++++++++++++
Coxsackievirus A10	19548	0.539	+
Human coxsackievirus B3	6691	0.185	+
Caulobacter phage Ccr29	2514	0.069	+
Enterovirus A71	2410	0.066	+
Coxsackievirus A14	766	0.021	+
Moraxella phage Mcat16	458	0.013	+
BeAn 58058 virus	290	0.008	+
Human coxsackievirus B5	222	0.006	+
Coxsackievirus A2	193	0.005	+
Human enterovirus 79	191	0.005	+

Threshold for detection

- at least 3 reads
- multiple sites in the genome
- controls cleans
- not in much higher numbers in other sample
- correct workflow (RNA/DNA)

Swiss-wide ring trial on viral metagenomics

- Four University Virology Institutes and Spiez Laboratory
- Seven different pipelines
- Spiked plasma samples, multiplexed virus reagent
- Wet-lab and bioinformatics part



A case of rare virus infection resolved by metagenomic sequencing

23-year old student from canton of Zurich

- Symptoms
  - Severe headache for 4 days
  - Scrotal pain for 1 day
  - Fever (38.0 °C)
  - Signs of meningeal irritation
  - CT scan normal
- Diagnosis: viral meningitis/encephalitis and epididymitis

- Vacation in Italy (Liguria) 2 weeks before

Diverse etiology for viral meningitis/encephalitis

Phenuiviridae	Bunyamwera virus California encephalitis virus Jamestown Canyon virus La Crosse encephalitis virus Rift Valley fever virus Toscana virus (TOSV)
Flaviviridae	Kyasanur forest disease virus Tick-borne encephalitis virus (TBEV)/FSME Japanese encephalitis virus Murray Valley encephalitis virus St. Louis encephalitis virus West Nile virus Banna virus Colorado tick fever virus
Togaviridae	Eastern equine encephalitis virus Western equine encephalitis virus Chikungunya virus
Enteroviren	Poliovirus Echovirus Coxsackiviruses Enteroviruses
Herpesviruses	HSV-1/2 HHV-6 EBV VZV CMV
Other viruses	Mumps Measles Rubella HIV Adenovirus Influenza A/B Parainfluenza Lymphocytic choriomeningitis virus Parvovirus B19 Rotavirus Vaccinia Encephalomyocarditis virus

Routine diagnostic results all negative

- Enterovirus PCR CSF negative
- Enterovirus PCR Blood negative
- HSV-1/2 PCR CSF negative
- VZV PCR CSF negative

- FSME IgM CSF negative
- EBV IgM Blood negative
- Measles IgM Blood negative
- Mumps IgM Blood negative
- Rubella IgM Blood negative
- HIV-1 Combo-Test negative

Liquor	
Enterovirus	
Enterovirus RNA-Nachweis, ql (RT-PCR)	negativ
Frühsommer-Meningoencephalitis	
FSME IgM, ql (ELISA)	negativ
Herpes-Simplex-Virus (HSV)	
HSV-1 DNA-Nachweis, ql (PCR)	negativ
HSV-2 DNA-Nachweis, ql (PCR)	negativ
Varizella-Zoster-Virus (VZV)	
VZV DNA-Nachweis, ql (PCR)	negativ

Viral Metagenomics detects Sandfly fever Naples phlebovirus virus in CSF

Domain level taxonomy profile

domain	reads	percent	abundance
bacterial	597	0.010	+
bovine	19	0.000	+
fungal	16	0.000	+
human	5710560	97.873	++++++++++++++++++++++
unknown	123032	2.109	+
viral	444	0.008	+

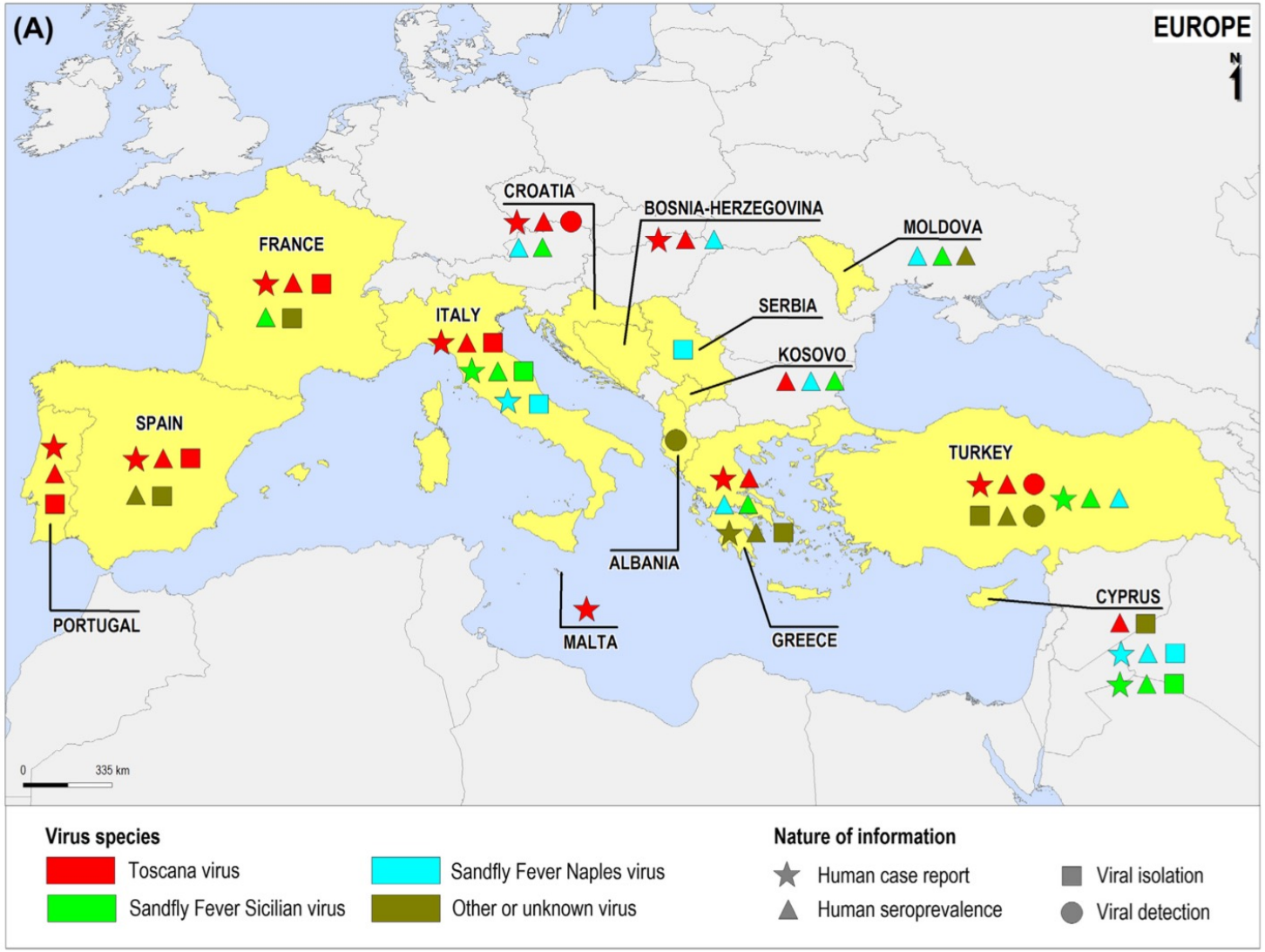
Relevant Virus species

species	1000414117-LI-DNA_S5	1000414117-LI-RNA_S10	reads_total
Sandfly fever Naples phlebovirus	0	284	284

Subtype Toscana virus (TOSV)

Sandfly fever Naples phlebovirus

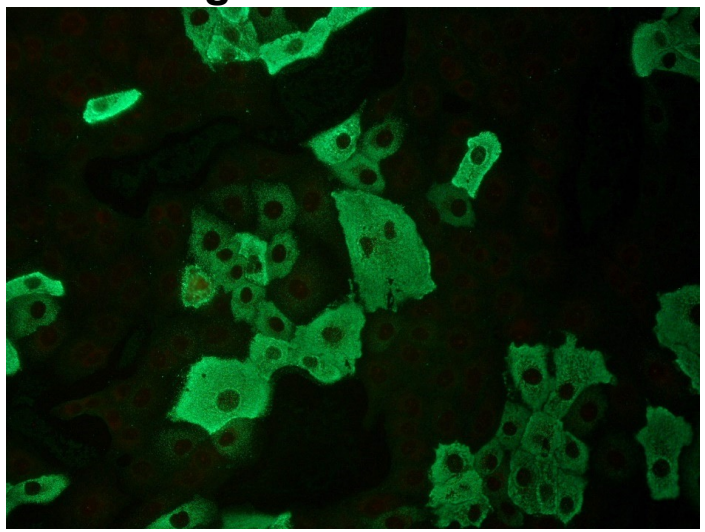
- Transmission by sandflies (*Phlebotomus*)
- Three subtypes
  - Siciliy (S)
  - Toscana (T)
  - Naples (N)
- Fourth published case of TOSV in Switzerland
- Unusual clinical manifestation of meningitis and epididymitis
- TOSV has to be considered in returning travellers



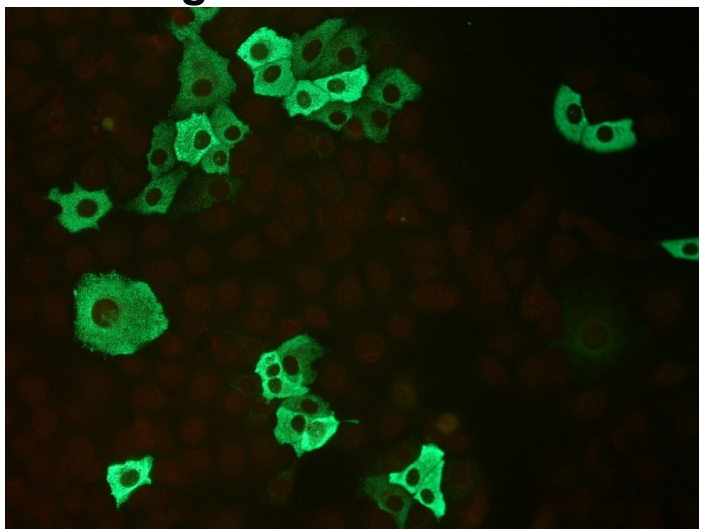
Alkan, 2013, Antiviral Research

Toscana virus confirmed by PCR and serology

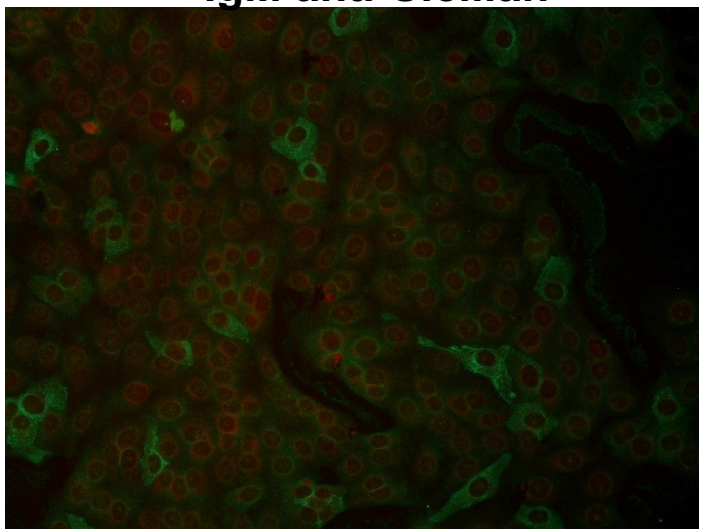
IgM anti-Toscana



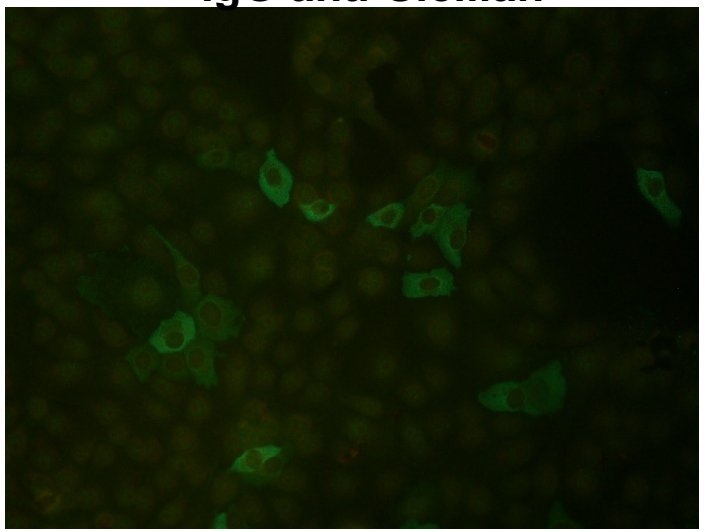
IgG anti-Toscana



IgM anti-Sicilian

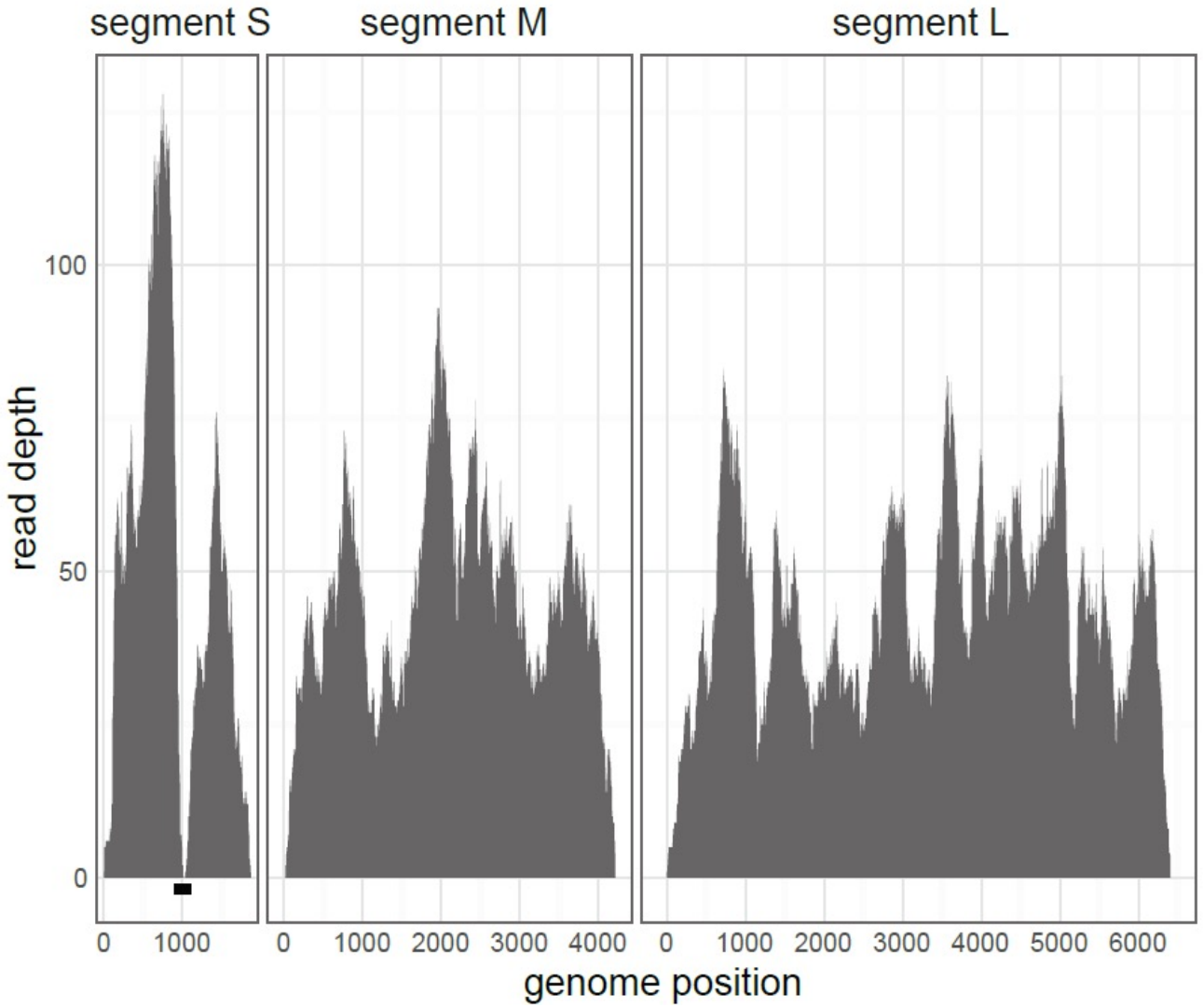


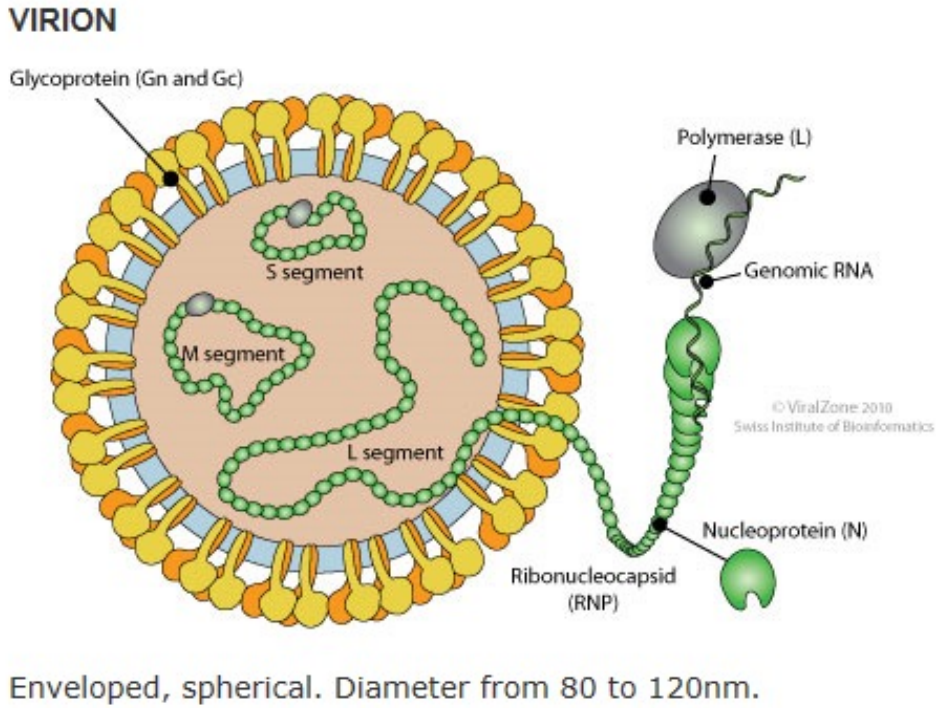
IgG anti-Sicilian



Fiona Pigny, Geneva

Full length genome sequence of Toscana virus obtained

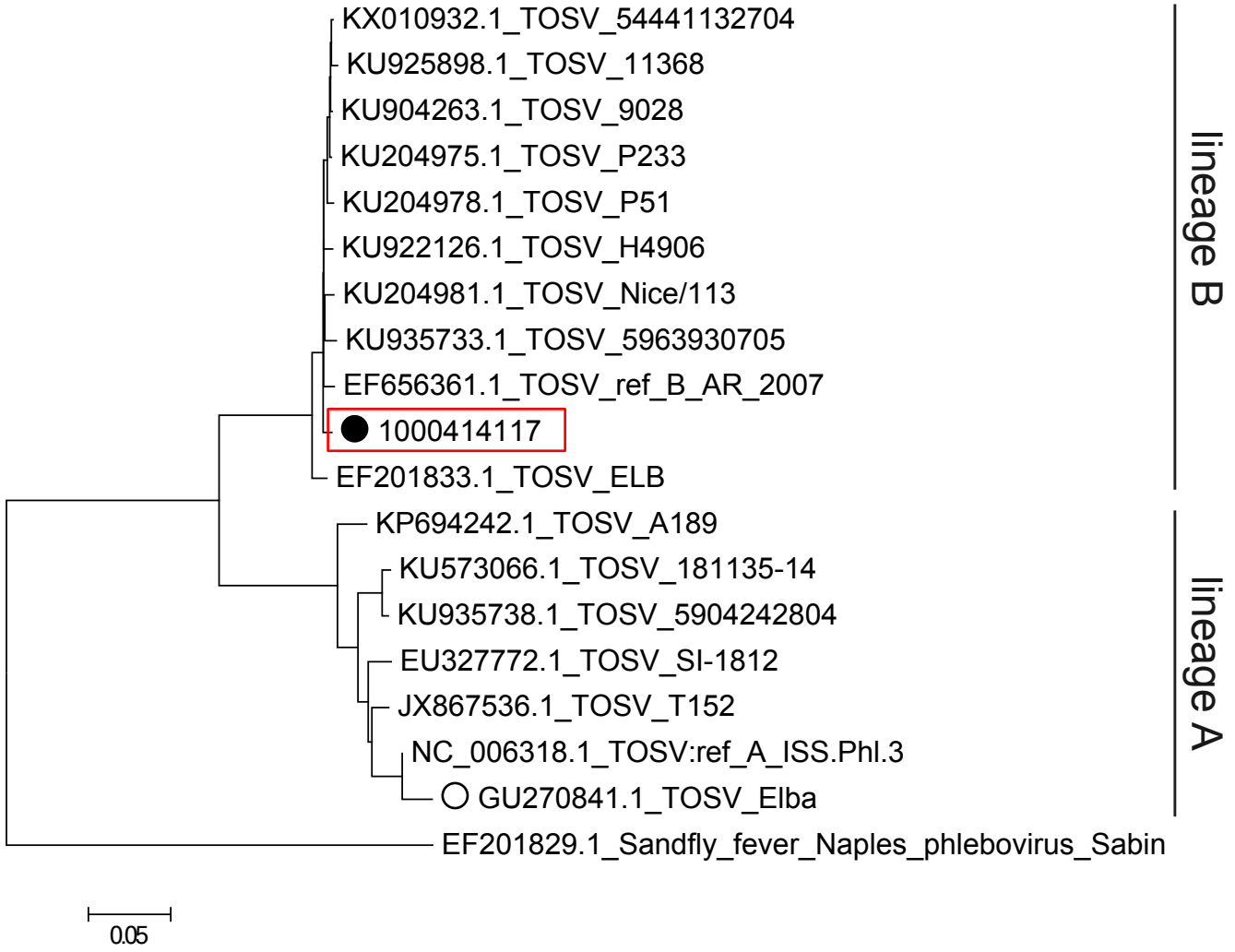




Enveloped, spherical. Diameter from 80 to 120nm.

Tschumi, BMC Infect Dis, 2019

Patient isolate belongs to Toscana virus lineage B



Tschumi, BMC Infect Dis, 2019

Conclusions Toscana virus case

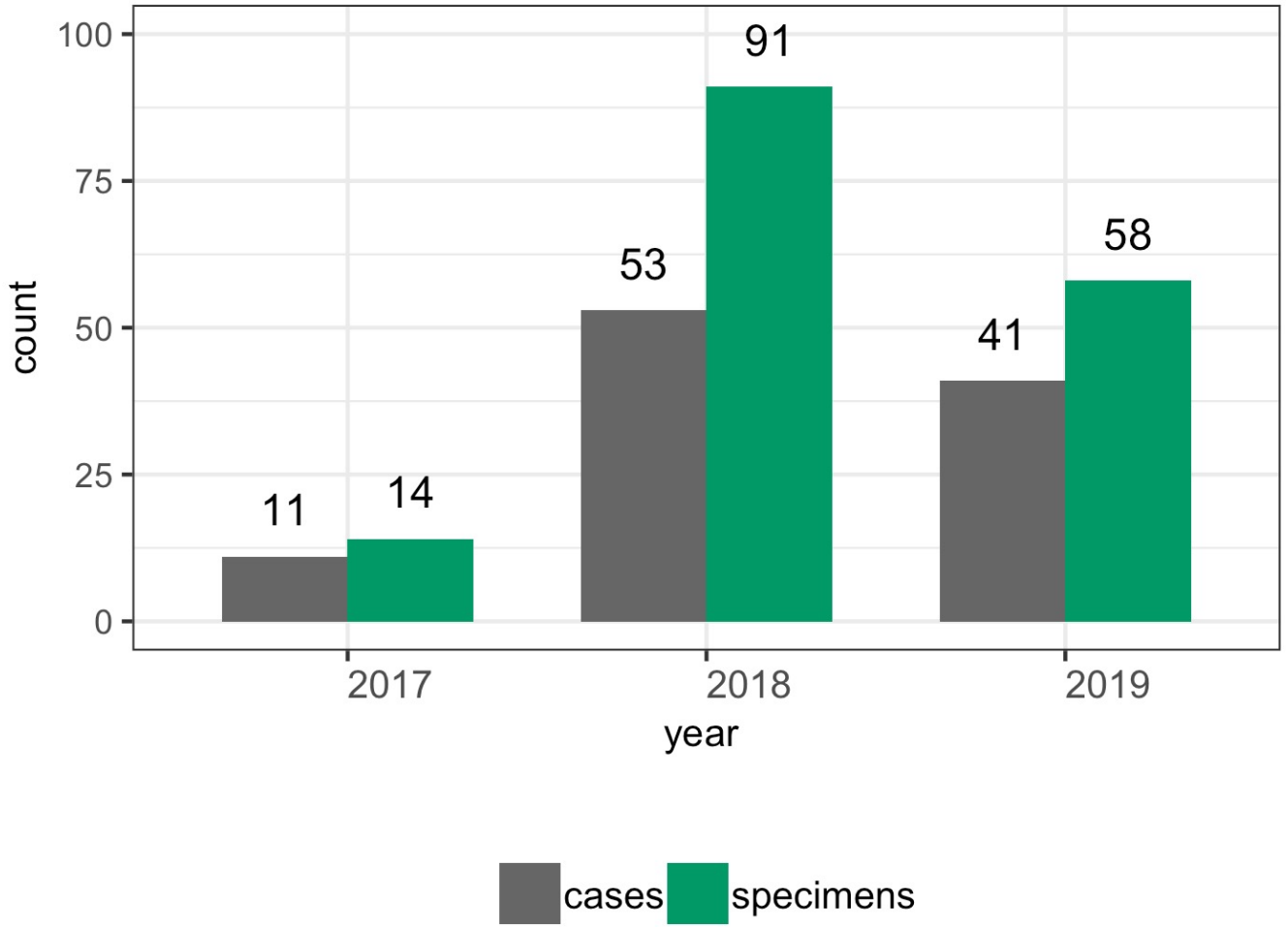
- Metagenomic sequencing delivered final diagnosis
- Direct impact on antibiotic treatment
- First report of lineage B Toscana virus in Italy

Two Years of Viral Metagenomics in a Tertiary Diagnostics Unit: Evaluation of the First 105 Cases

Two years of viral metagenomics in a tertiary diagnostics unit: evaluation of the first 105 cases

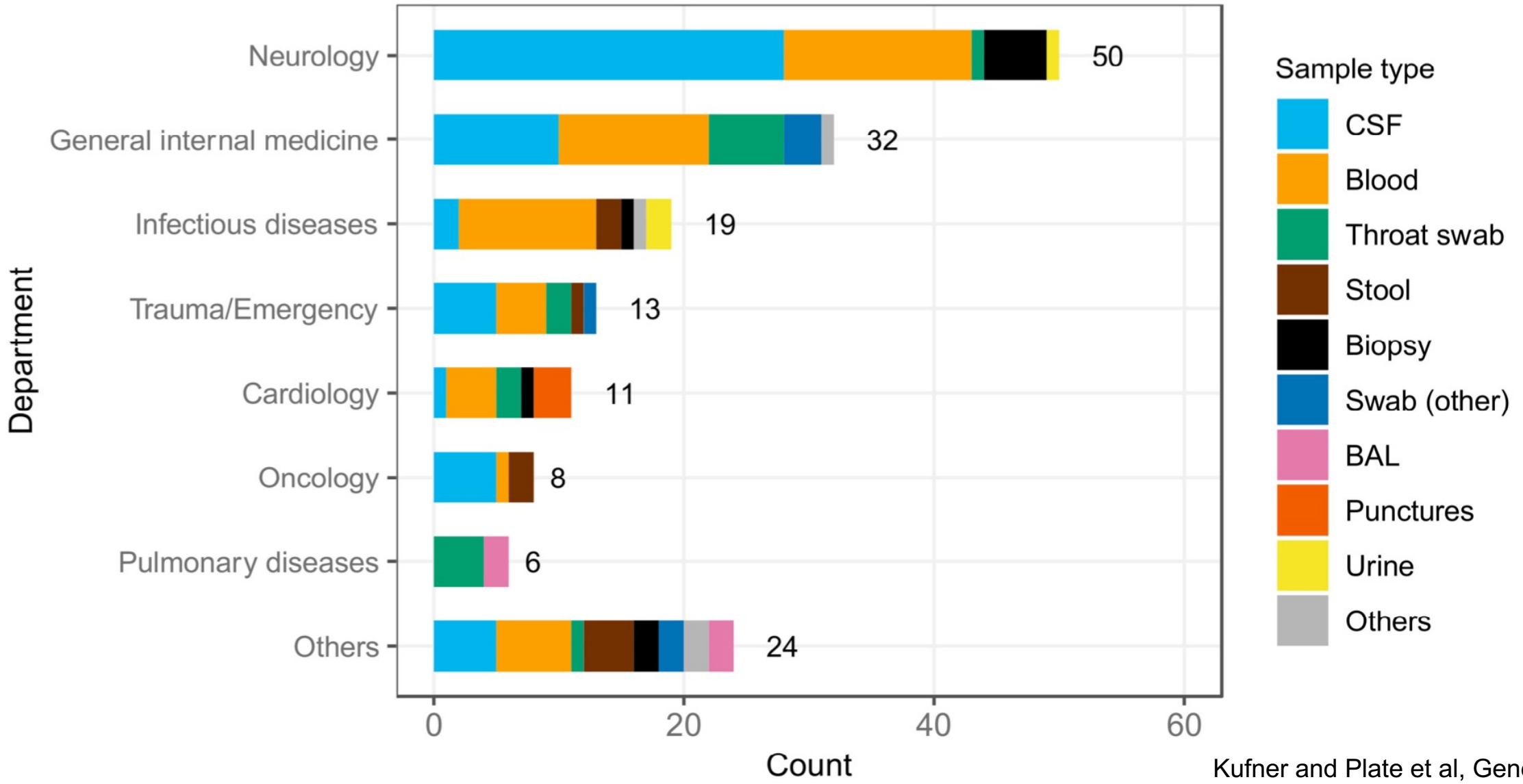
Viral metagenomic sequencing of 105 cases (2017–2019)

- Unknown etiology of infection even after extensive conventional testing
- Very broad initial differential diagnosis
- Analysis requested by treating physician and ID consultant service of USZ



Kufner and Plate et al, Genes, 2019

CSF samples from neurology department most frequent



Kufner and Plate et al, Genes, 2019

Large range of different viruses detected

- 34 out of 105 cases (32%) positive
- 27 distinct virus species belonging to 13 virus families
- Anelloviruses, Flaviviruses and Herpesviruses found most frequently
- In one third of specimen multiple viruses detected

Kufner and Plate et al, Genes, 2019

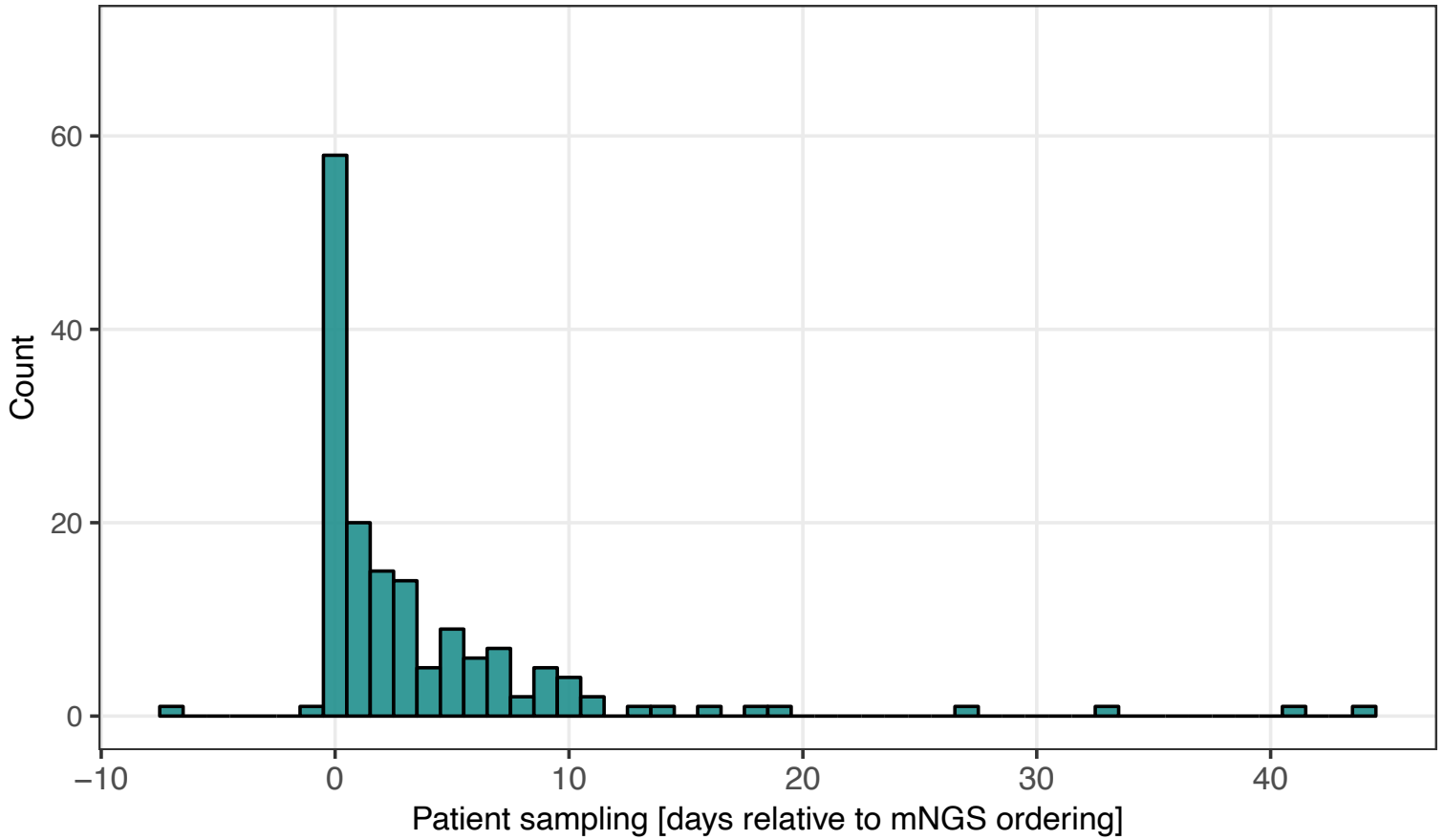
Outcome of mNGS vs Conventional Testing

- Evaluate the potential of mNGS to detect virus infections that were found with the respective conventional test
- Good concordance with standard clinical testing

PPA = positive percent agreement (sensitivity)
NPA = negative percent agreement (specificity)
OPA = overall percent agreement

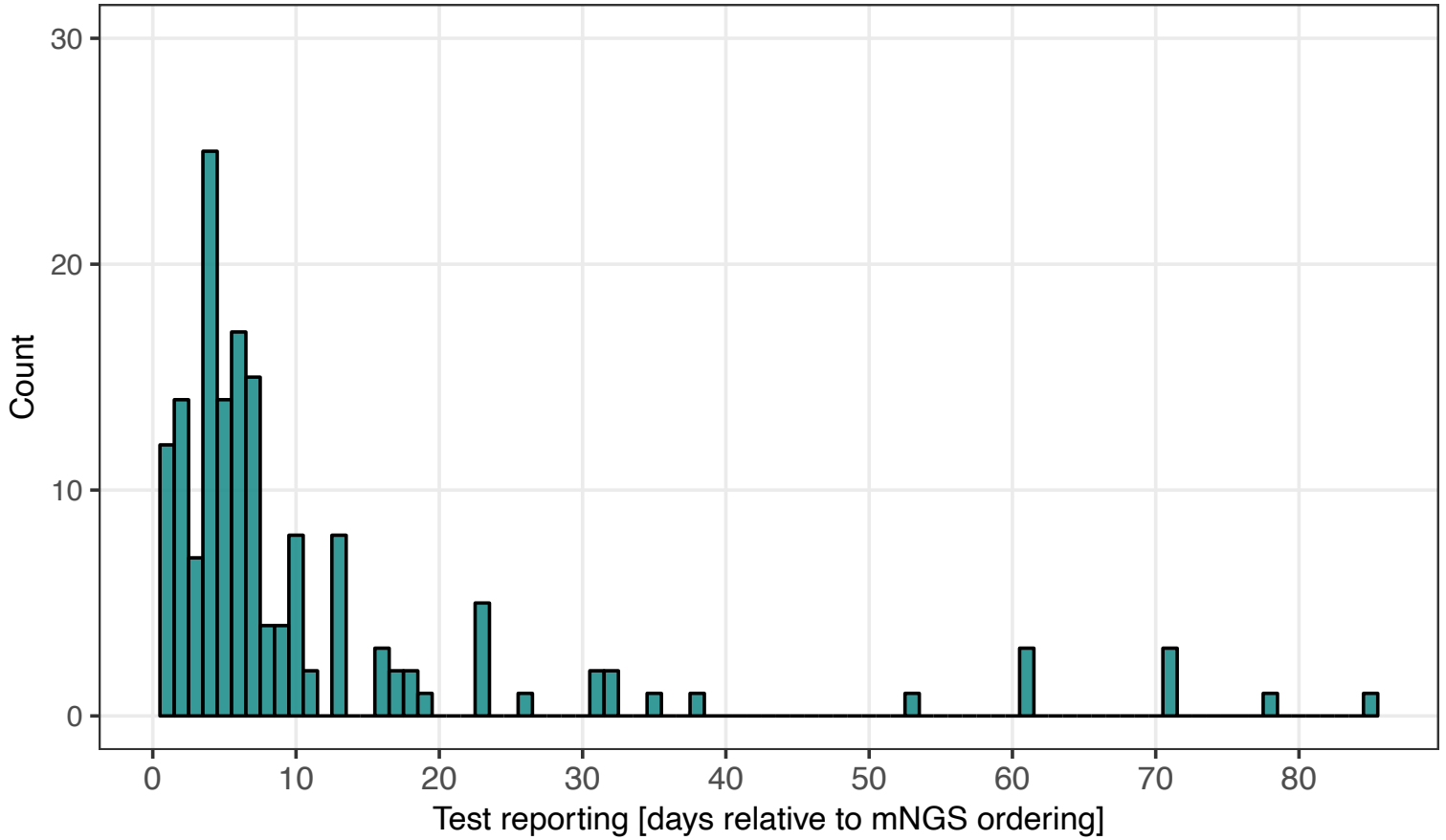
				Respective conventional testing +	Respective conventional testing -
All Samples	OPA = 94%	PPA = 65/92%	mNGS +	22	2
		NPA = 95%	mNGS -	2 pos 10 low pos	39
CSF	OPA = 91%	PPA = 64/88%	mNGS +	7	1
		NPA = 93%	mNGS -	1 pos 3 low pos	14
Blood	OPA = 100%	PPA = 46/100%	mNGS +	5	0
		NPA = 100%	mNGS -	0 6 low pos	8
Throat swab	OPA = 91%	PPA = 100%	mNGS +	4	1
		NPA = 86%	mNGS -	0	6

Days between patient sampling and mNGS ordering



- mNGS most often requested at the day of patient sampling
- Some cases where mNGS is only ordered at a later timepoint

mNGS Turnaround Time



- In most cases reported to the clinician within 7 days (65.4%)

Study Subgroup

- Study subgroup with informed consent (*n* = 67)

→ Retrospective analysis of clinical charts to determine clinical impact

Age: median (range)	53 (17 – 88 years)
Male gender	43 (64.2%)
Patients immunocompromised	24 (35.8%)
Post SOT	7 (29.2%)
Malignancy	5 (20.8%)
HIV	5 (20.8%)
Autoimmune disorder	7 (29.2%)
Department	
Internal medicine and subspecialties	35 (52.2%)
General internal medicine	15 (22.4%)
Cardiology	7 (10.4%)
Infectious diseases	7 (10.4%)
Pulmonolgy	3 (4.5%)
Rheumatology	2 (3%)
Hematology / Oncology	1 (1.5%)
Neurology / Neurosurgery	28 (41.8%)
Neurology	26 (38.8%)
Neurosurgery	2 (3%)
Other	4 (6%)
Emergency department	1 (1.5%)
Otorhinolaryngology	1 (1.5%)
Dermatology	2 (3%)

Study Subgroup
– Tentative diagnosis when test was ordered

mNGS most often ordered in patients with

- meningitis and/or encephalitis
- peri-/myocarditis
- febrile syndromes

Disease	Nb of cases
Neurological disorders	
Meningitis and/or encephalitis	17
Other central nervous system disorders [1]	11
Cerebral lesion/abscess	3
Peripheral nervous system disorders	2
PML	1
Other diseases, disorders & syndromes	
Pericarditis and/or myocarditis	8
Febrile syndromes (including FUO)	8
Respiratory tract infections	4
Allograft dysfunction after lung transplantation	3
Diarrhea	3
Sepsis in neutropenia	1
Cytokine-Release-Syndrome	1
Unspecific polyarthritis and lymphadenopathy	1
Constitutional symptoms unknown etiology	1
Unspecific myalgia syndrome	1
Unspecific cutaneous lesions	1
Chronic sinusitis	1

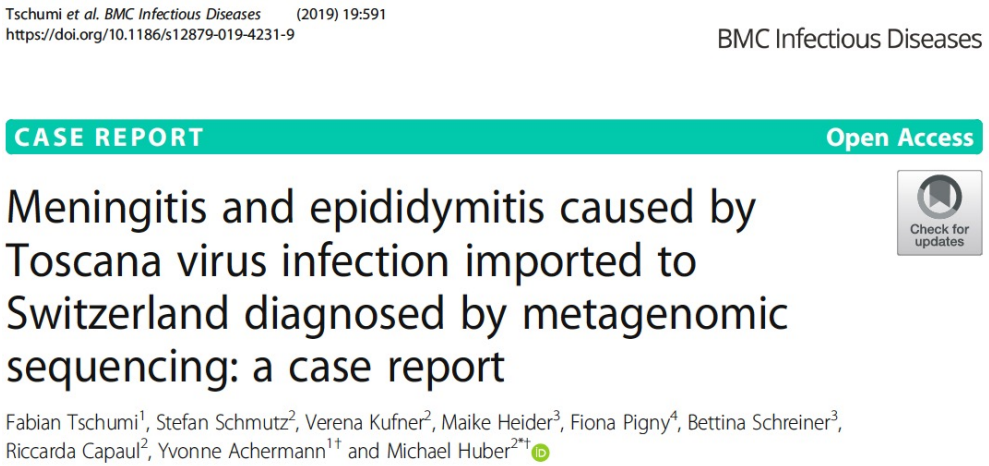
Metagenomic sequencing provided final diagnosis in three cases

Final diagnosis by metagenomic sequencing

- Sandfly fever Naples phlebovirus associated meningitis
- Unexpected Tick-borne encephalitis virus
- Pegivirus C associated meningoencephalitis

“Exclusion” of viral infection in two cases

- negative sequencing results contradicting a viral infection, although not completely excluding, allowed to increase immunosuppression


Tschumi *et al. BMC Infectious Diseases* (2019) 19:591
https://doi.org/10.1186/s12879-019-4231-9

BMC Infectious Diseases

CASE REPORT Open Access

Meningitis and epididymitis caused by Toscana virus infection imported to Switzerland diagnosed by metagenomic sequencing: a case report

Fabian Tschumi[1], Stefan Schmutz[2], Verena Kufner[2], Maike Heider[3], Fiona Pigny[4], Bettina Schreiner[3], Riccarda Capaul[2], Yvonne Achermann[1†] and Michael Huber[2*†]

Kufner and Plate et al, Genes, 2019

Clinical impact of metagenomic sequencing

- Helpful or even decisive diagnostic tool in a few cases
- Advantages of an untargeted approach to complement routine tests
- Cave: study set with highly diverse clinical histories, bias for difficult-to-diagnose cases

Clinical impact of metagenomic sequencing in encephalitis/meningitis

- Identification of a viral cause in **13%** of encephalitis, meningoencephalitis, and meningitis cases (Zanella et al., Clin Microbiol Infect, 2019)
- In **28 of 44** cases novel, rare or unexpected viruses were detected (Brown et al., J. Infection, 2018)

Acute upper respiratory tact infections in primary care

Acute upper respiratory tact infections in primary care

Acute upper respiratory tact infections

- Common reasons for consultation
- Etiology is predominantly viral (Rhinovirus, Coronavirus, Adenovirus)
- Diagnosis based on a clinical assessment
- Specific testing only in selected situations (immunocompromised, outbreak)
- Symptom-based therapy. Antiviral and antibiotic treatment is rarely indicated.

Consequences of inappropriate antibiotic prescription

- Antibiotic resistance
- Increased morbidity and mortality
- Increased costs
- Inappropriate antibiotic prescription in the US is estimated to be 50%

Evaluate the potential of viral metagenomic sequencing as a diagnostic tool in primary care for immunocompetent patients suffering from a respiratory tract infection

Study Design

Study Design

- Prospective cross-sectional study
- October 2019 – November 2020

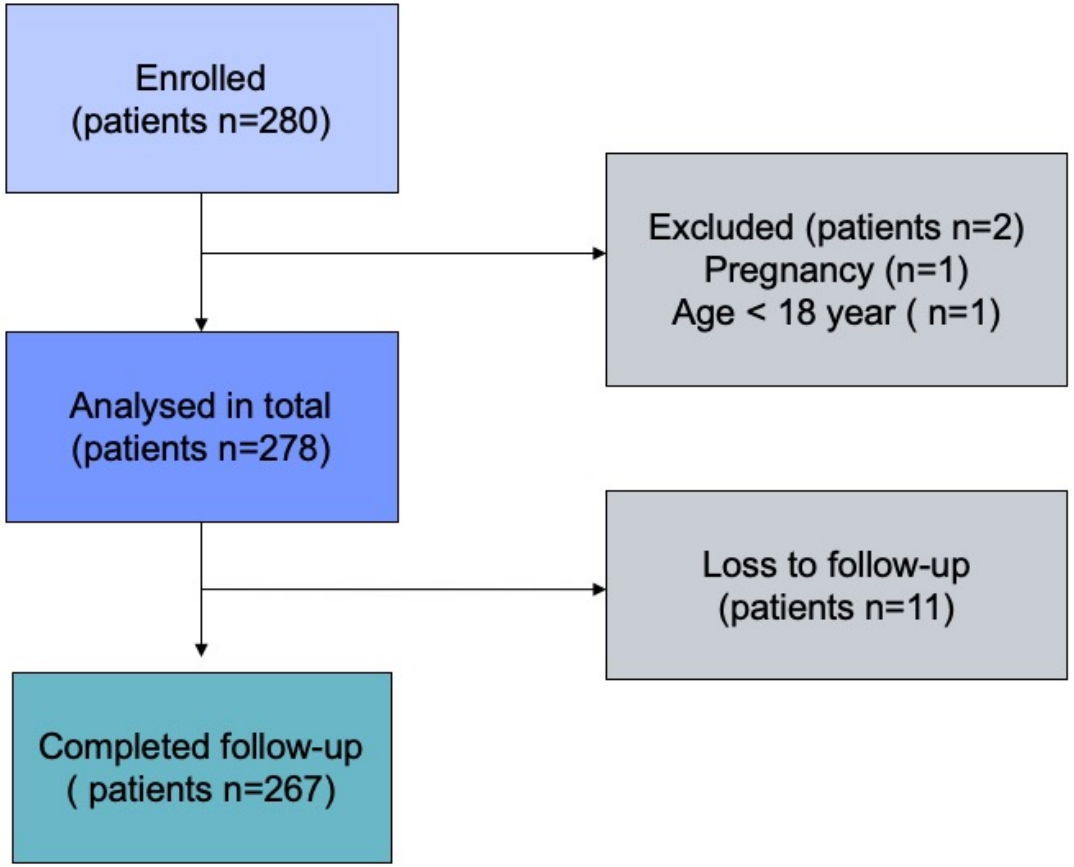
Eligibility and Recruitment

- 21 General Practitioners (ZH, BL)

Inclusion criteria

- Age > 18 years
- Clinically suspected respiratory tract infection
- Immunocompetent

Patient characteristics

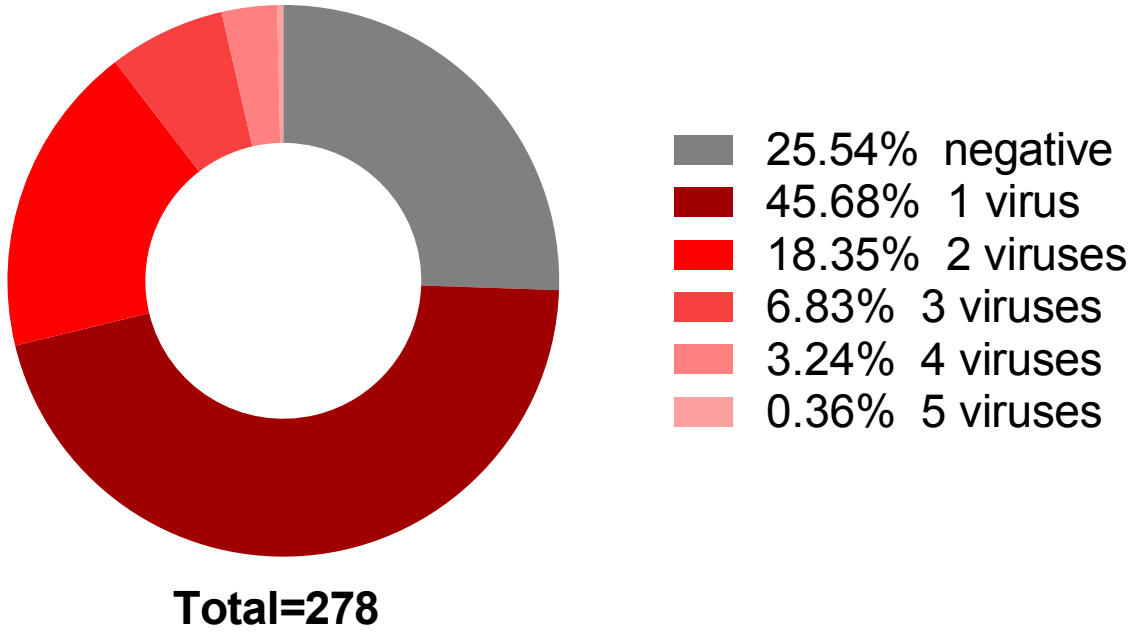


Patient characteristics

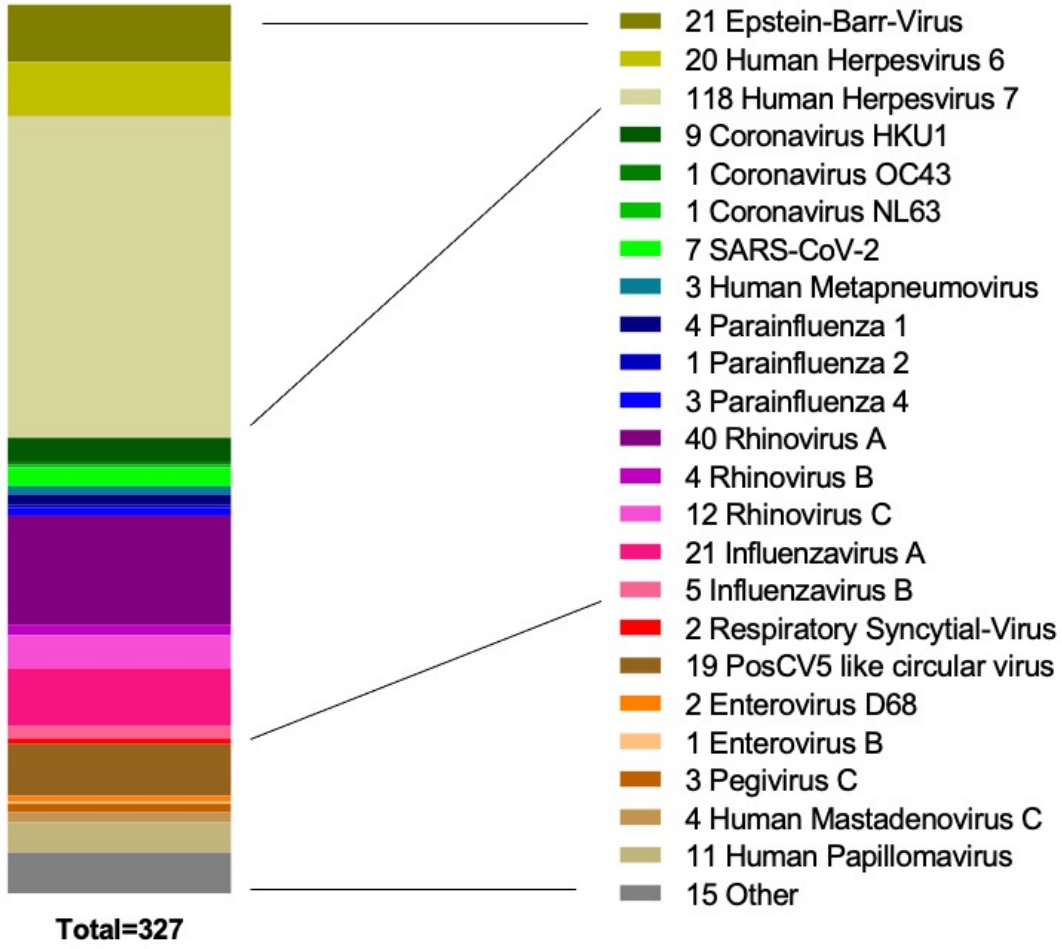
- Male (n/%): 119 (42)
- Age (median) in years: 39
- Fever (> 38.3C)
  - Measurement performed (n/%) 241 (86)
  - Fever (> 38.3C) (n/%): 12 (5)
- Influenza vaccination (n/%): 35 (12)

Preliminary Results

Detected viruses per patient

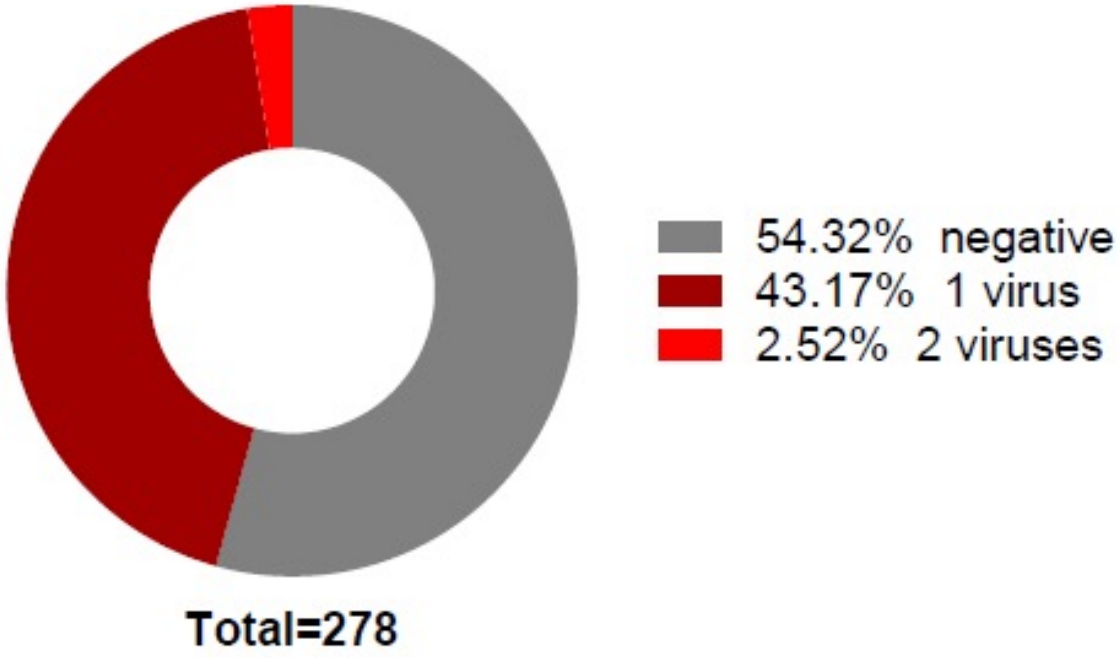


Differentiation of detected viruses

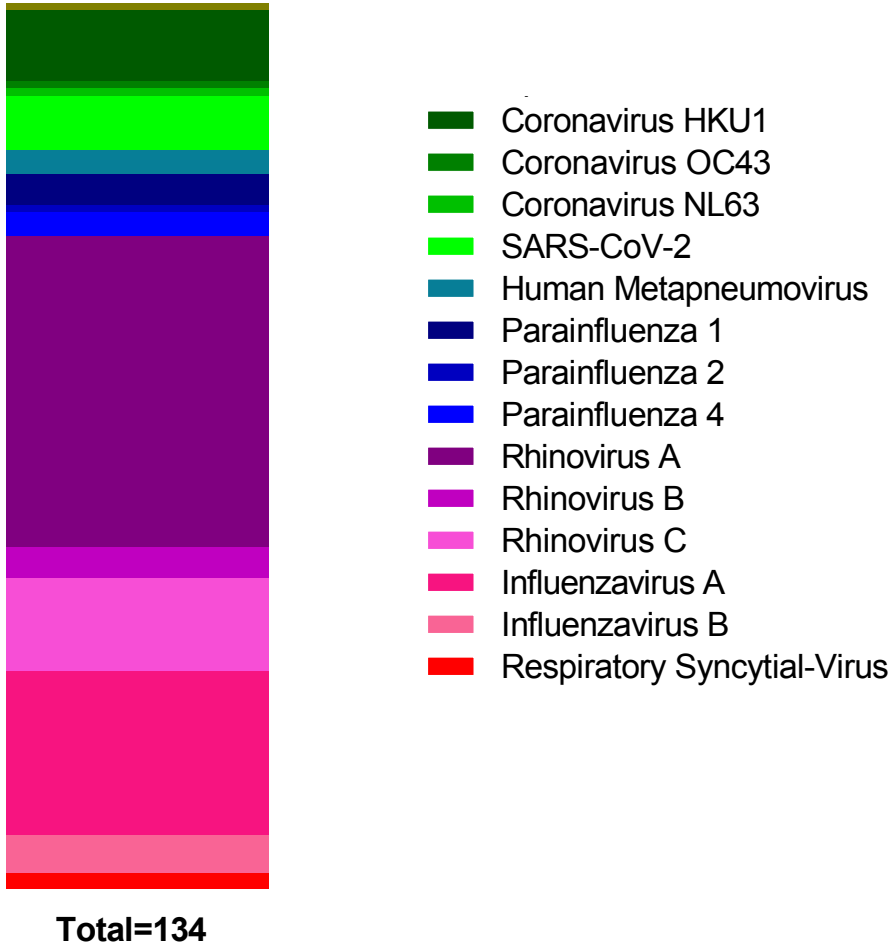


Preliminary Results “Respiratory Viruses”

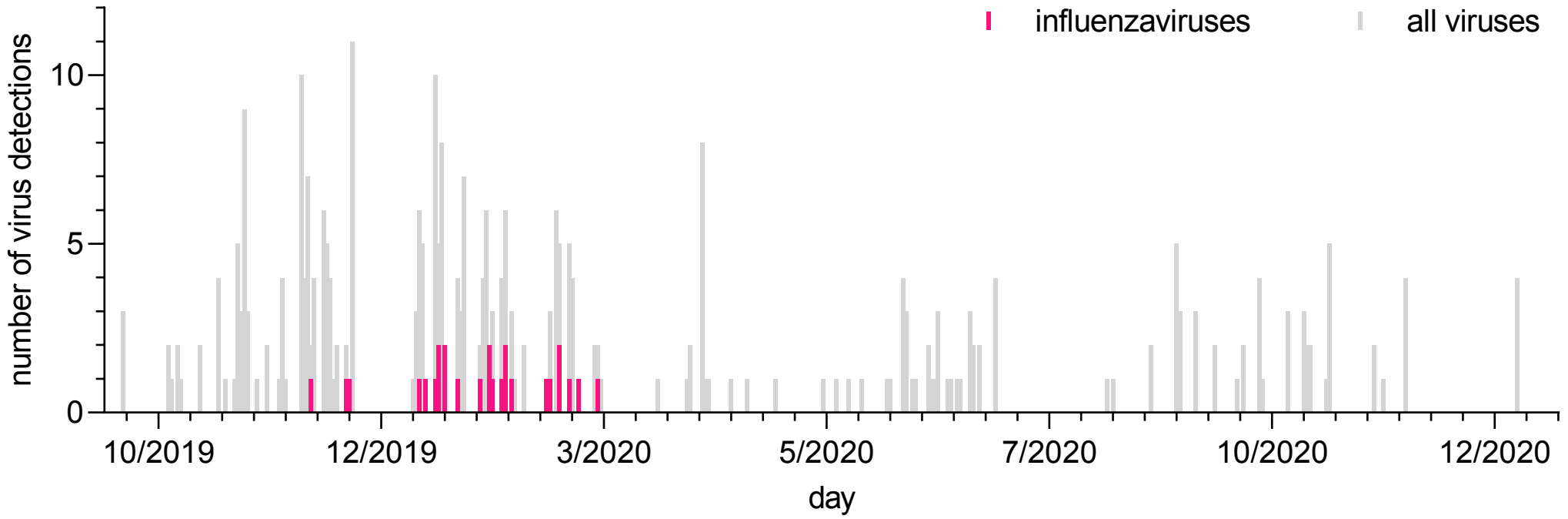
Detected “respiratory viruses” per patient



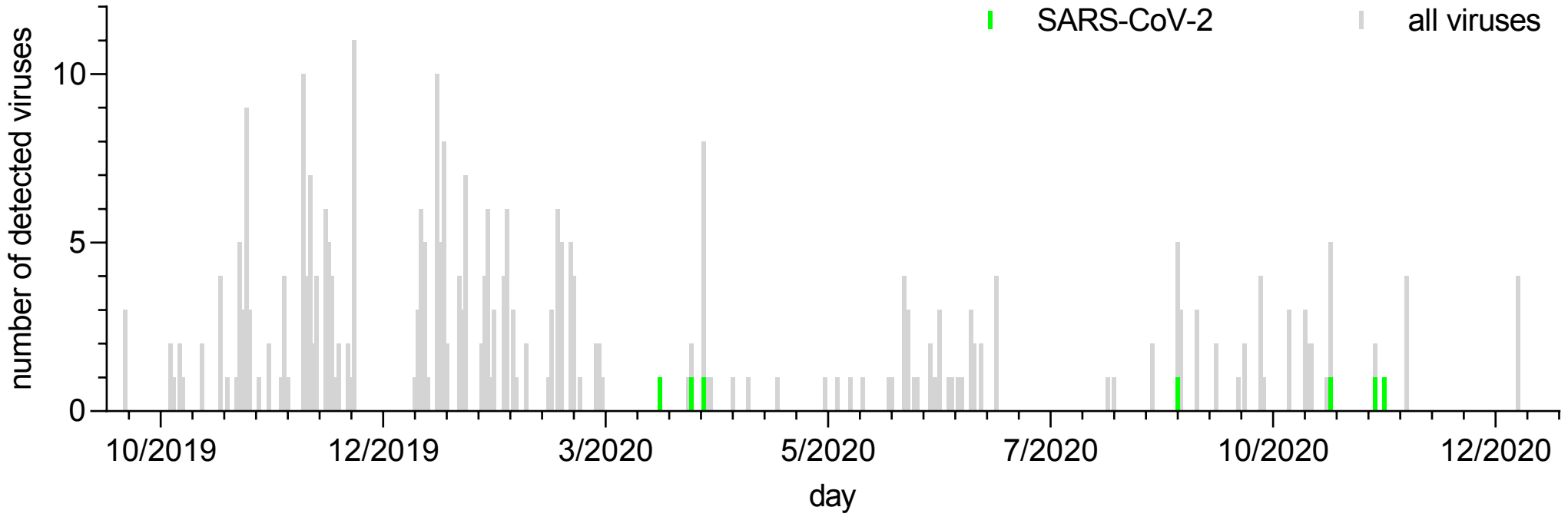
Differentiation of detected “respiratory viruses”



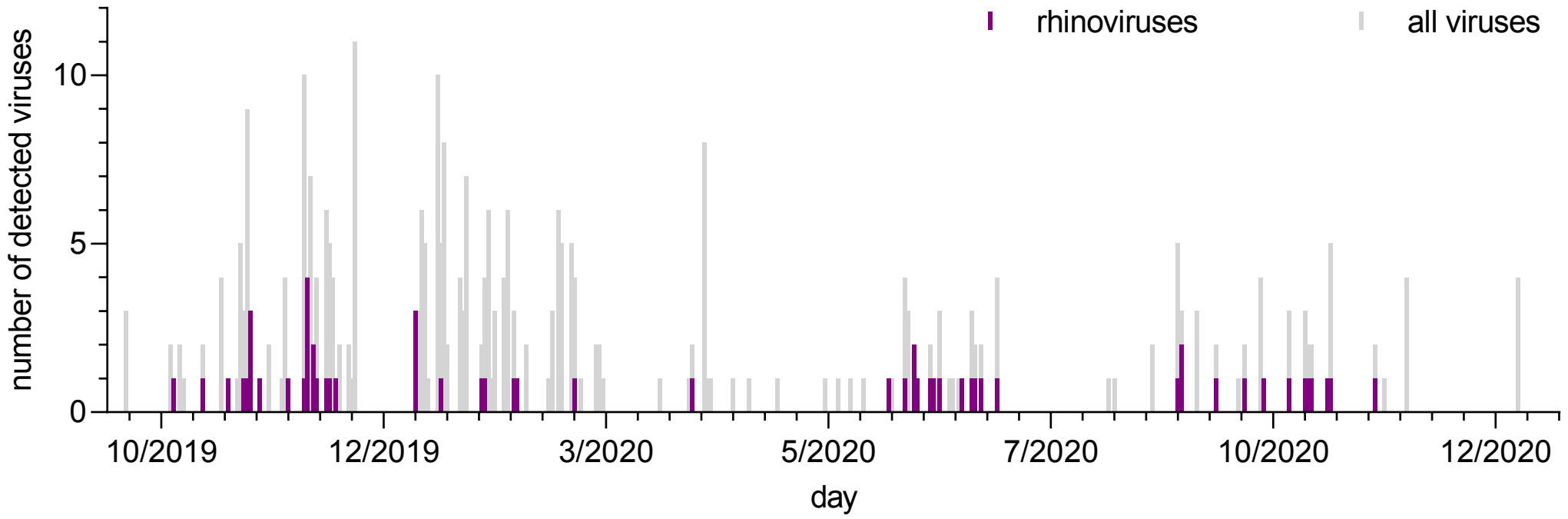
Timeline of viral detection – Influenzavirus



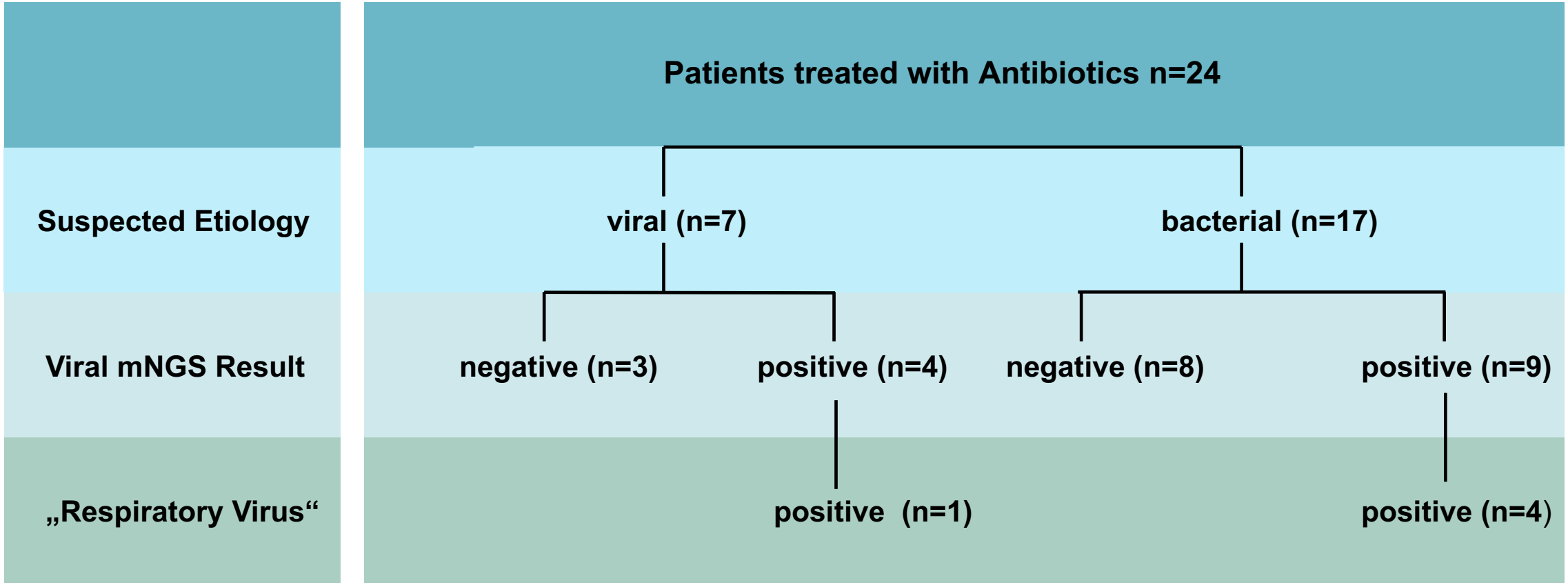
Timeline of viral detection – SARS-CoV-2



Timeline of viral detection – Rhinovirus



Preliminary Results Antibiotic Treatment



Outlook

Metagenomic virus sequencing today

- **Unbiased detection of viruses** not detectable/accounted for in routing diagnostics
- Proven benefit to **complement routine tests** in challenging diagnostic situations or infections with unknown etiology
- **Replaces multiple testing**
- Additional information on **virus genome**/genotype

Challenges for clinical application of metagenomic sequencing

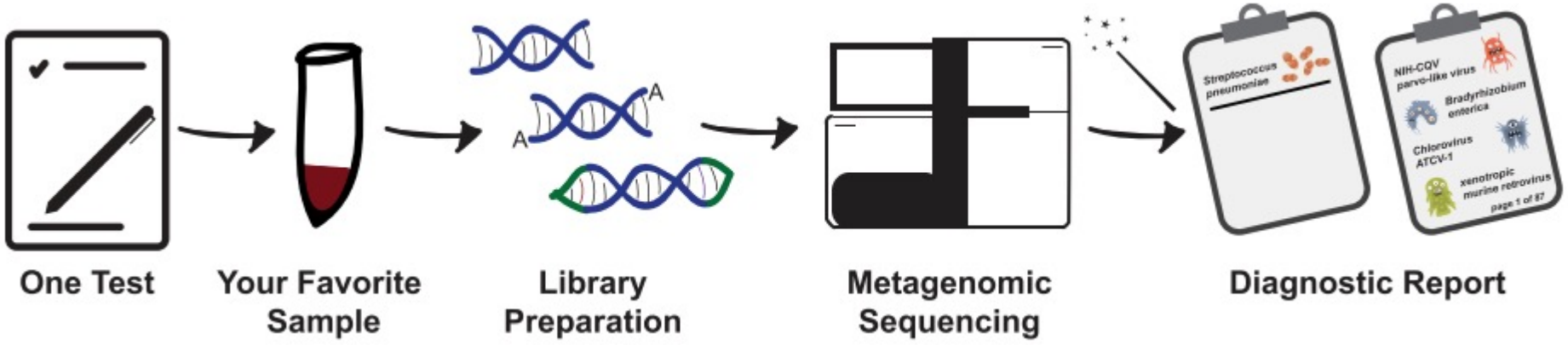
- Expensive, technically demanding, data analysis, bioinformatics
- Long turnaround time → rapid real-time Nanopore sequencing
- Less sensitive → capture probes, spiked primer enrichment, syndromic panels
- Contamination by sample/reagents/databases → controls, curated databases
- Comprehensive validation for all organism not possible → EQA, ring trials
- Differentiation of pathogenic organisms from commensals/background

  → Interdisciplinary collaboration with clinicians

The Future of Metagenomic Sequencing

- One test fits all
  - microbial metagenomics
  - virus strains
  - bacterial typing
  - resistance genes

- Broad, rapid and inexpensive first line diagnostic tool for selected patient groups or scenarios



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