genes

Special issue on “Viral Diagnostics Using NGS”

MDPI

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

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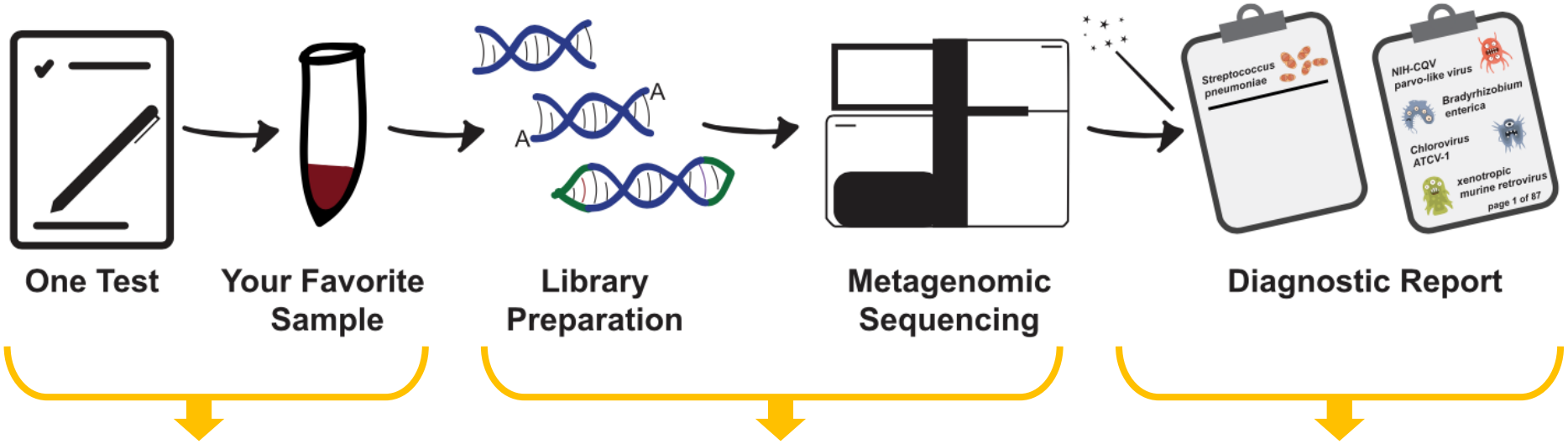
Diagnostic Metagenomics



CHALLENGES

- Access to mNGS testing
- Upfront or second-line testing
- Human host background
- Sample stability and transport
- Contamination
- Standardized clinical laboratory protocols
- Universally accepted reference standards

- Quality control metrics
- Workflow complexity
- Cost
- Turnaround times
- Sequence quality

- Computational power
- Misaligned sequences
- Database misannotations and biases in representation of organisms
- Bioinformatics software validation
- Patient privacy
- Medical reimbursement
- Clinical interpretation/utility/indications

Greninger et al., *Expert Review of Molecular Diagnostic*, 2018
Chiu, Miller, *Nature Reviews*, 2019

Viral Metagenomics Workflow

Wet lab

- Centrifugation
- Filtration (0.45 µm)
- Storage - 80°C
- Nucleic acid extraction
- DNase treatment

DNA	RNA
6N	6N
--	RT
--	RNase H treatment
2nd strand synthesis	2nd strand synthesis

Library preparation NexteraXT

Sequencing

Illumina MiSeq

- read length: 150 bp
- 16 h run time
- ~ 6.8 M reads/sample

Kufner, Plate et al., *Genes*, 2019
https://github.com/medvir/virome-protocols

Dry lab

VirMet

- FASTQ files
- Quality filter
- Decontamination
- BLAST against viral database
- Virus species

https://github.com/medvir/VirMet

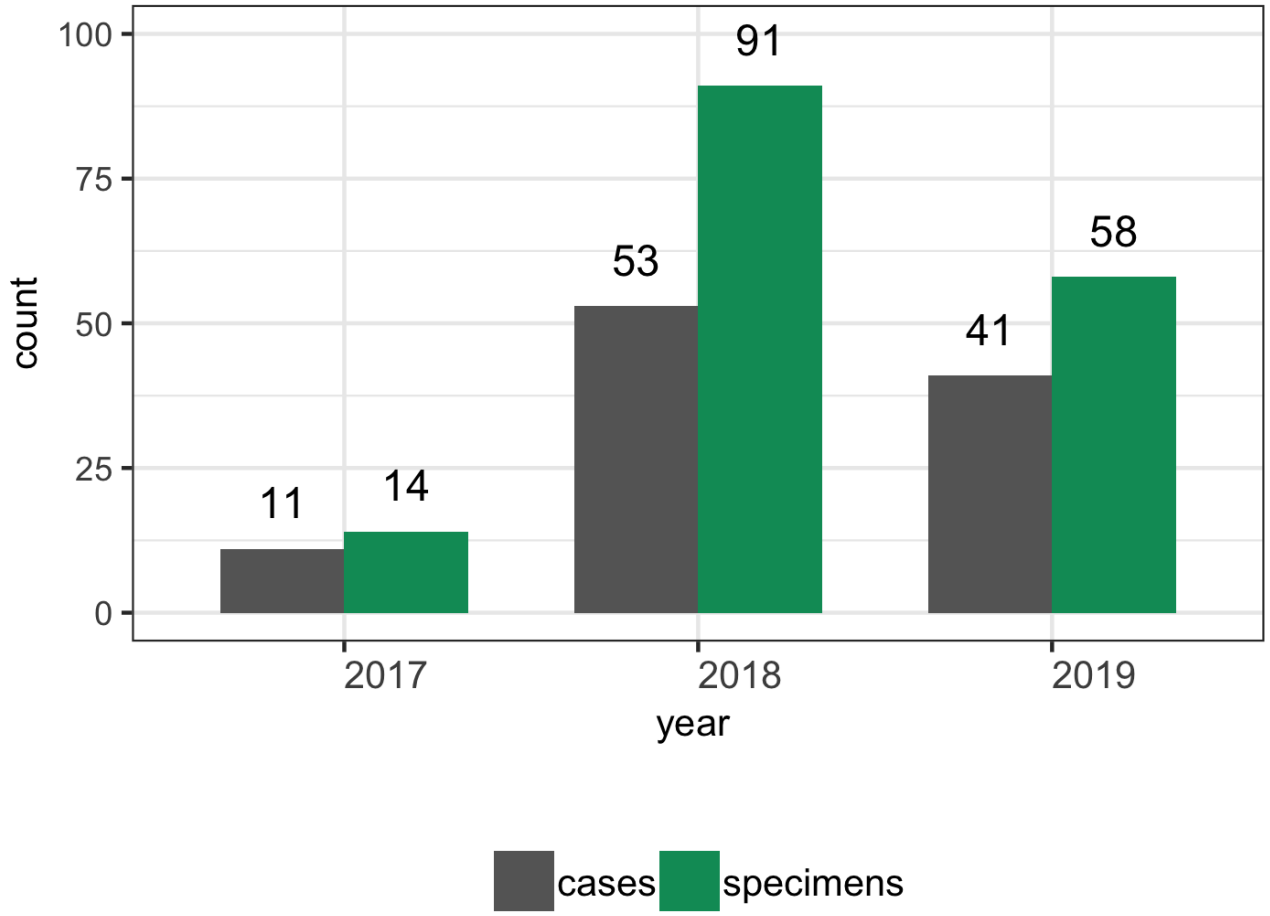
Aim

Evaluate clinical utility and impact of viral metagenomics on diagnosis

Comparison of **outcome** and **workload** to conventional routine testing

Study Design

- All performed mNGS assays on clinical cases analyzed between May 2017–June 2019 (*n* = 105)
- Criteria for performing test:
  - ✓ Unknown etiology of infection even after extensive conventional testing
  - ✓ Very broadly formulated initial differential diagnosis
- Analysis requested by treating physician or infectious disease consultant service of USZ



Study Design - 2

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

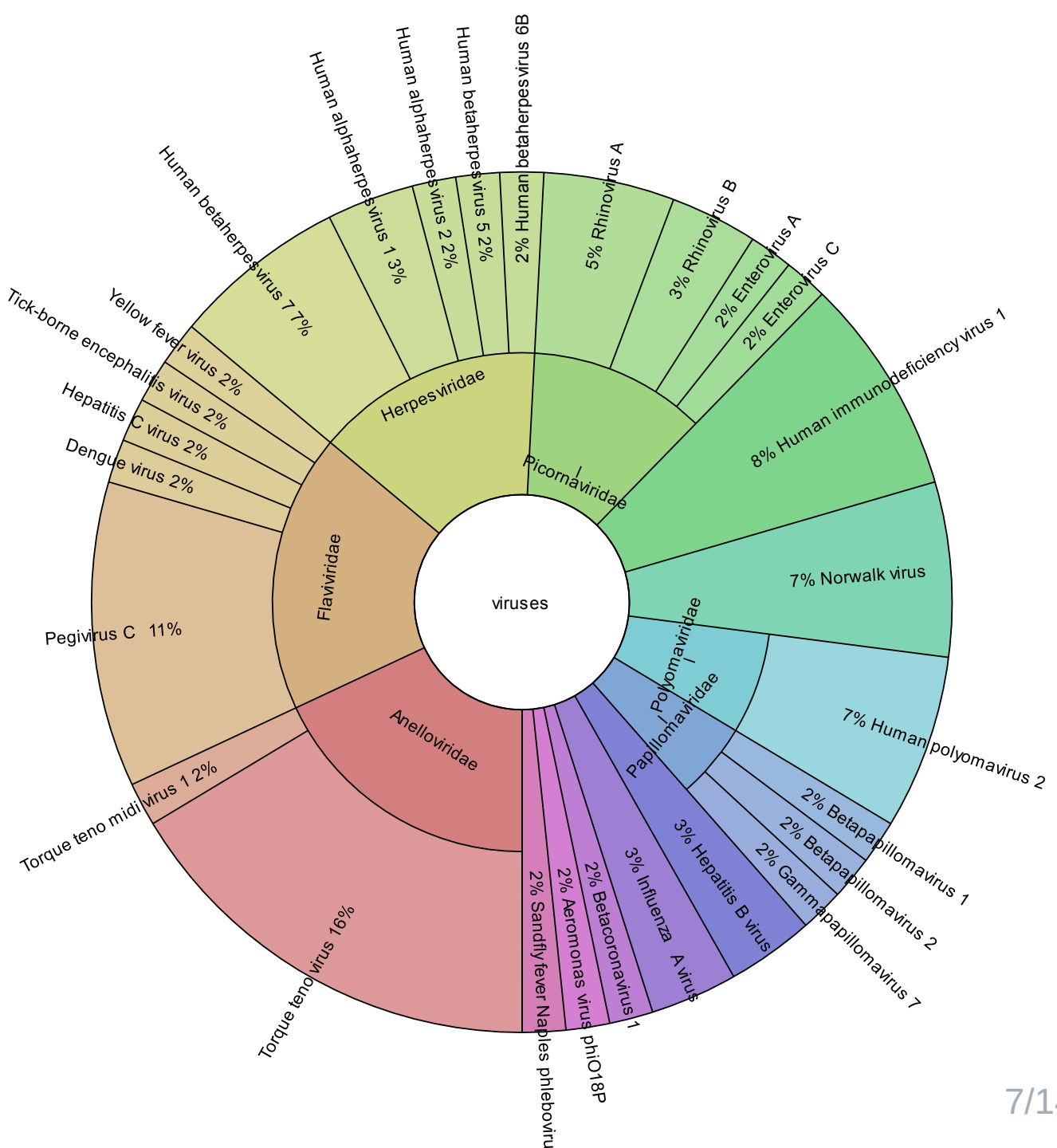
→ Retrospective analysis of clinical charts to determine clinical impact

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%)

Detected Viruses

- 34 positive cases (32%)
- Reported 27 distinct virus species belonging to 13 virus families
- Anelloviruses found most frequently (21%)
- In 11 cases (32.4%) mNGS detected **multiple viruses per specimen**

  → 1.4 detected viruses over all cases



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**

			Respective conventional testing +	Respective conventional testing -
All Samples	PPA = 65/92%	+	22	2
OPA = 94%		mNGS		
	NPA = 95%	-	2 pos 10 low pos	39

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

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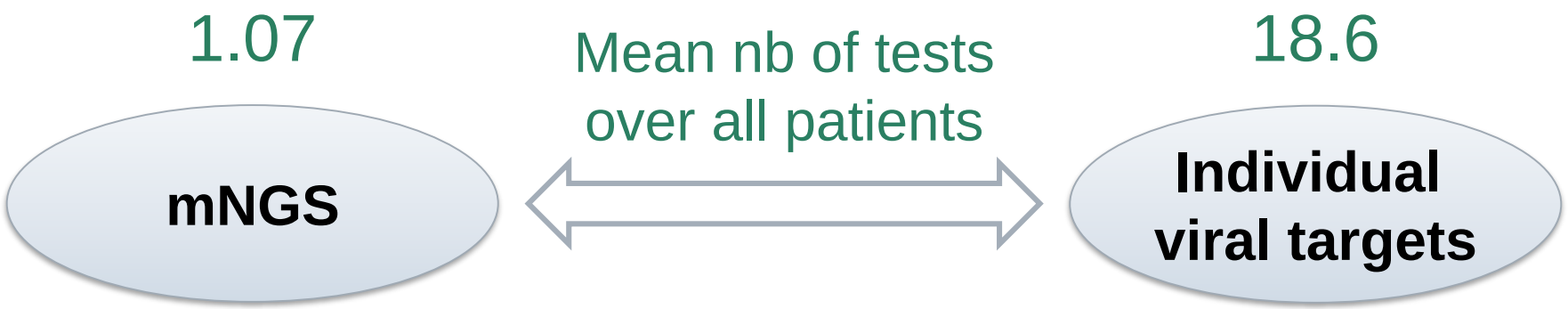
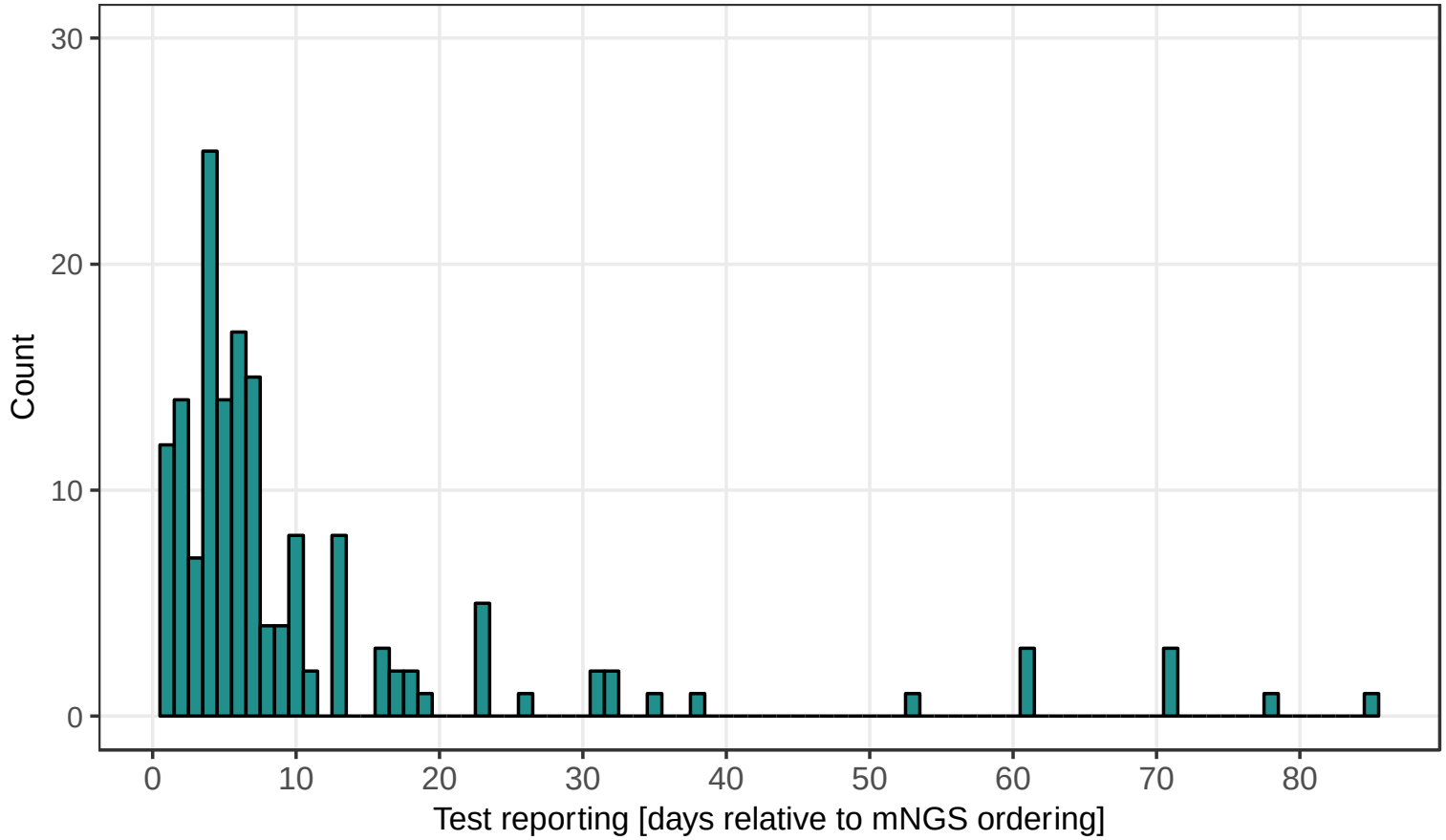
Family (-viridae)	PPA [%]
Flavi	75/100
Herpes	15/50
Picorna	100
Polyoma	100
Retro	100
Rest	100

Workload of mNGS vs Conventional Testing



Timing of mNGS
– days between mNGS ordering and reporting



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

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

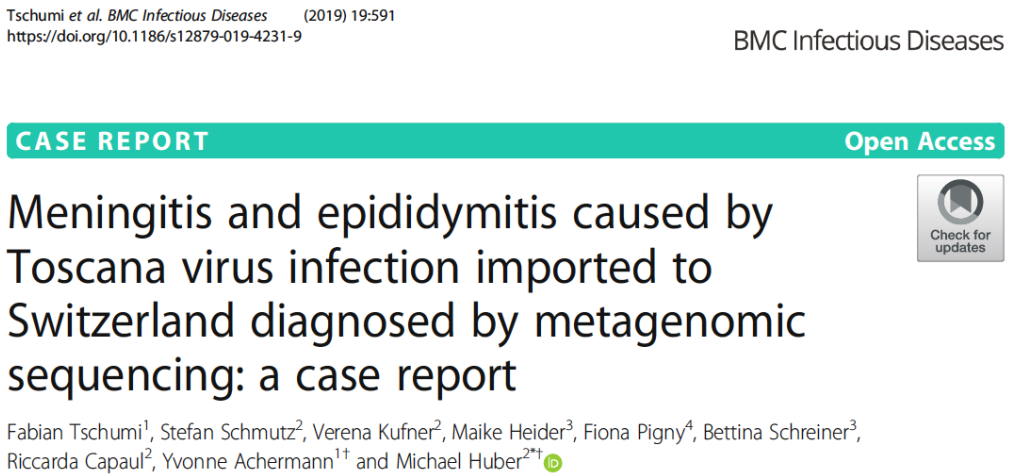
Clinical Impact of mNGS

- In three cases mNGS provided the final diagnosis:

→ Sandfly fever Naples phlebovirus associated meningitis

→ Tick-borne encephalitis

→ Pegivirus C associated meningoencephalitis



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*†]

- In one heart transplant recipient, treatment was adjusted after confirmation of positive routine test (Norovirus) and exclusion of further viruses

- A patient with inflammatory central nervous system disorder was treated with high-dose immunosuppression after exclusion of viral infection

Summary Two Years of mNGS

- Evaluation of a study set with highly diverse clinical histories
- In some cases viral metagenomics proved as helpful or even decisive diagnostic tool
- Mainly used for difficult-to-diagnose cases where routine tests remain negative → potential bias towards patients where no diagnosis is made
- Demonstrated the advantages of an untargeted approach
- Demonstrated the potential of mNGS to complement current routine tests
- Interdisciplinary collaboration with clinicians is key to put findings into clinical context

genes https://doi.org/10.3390/genes10090661

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