

Sequence-based typing of hepatitis A virus for multi-country outbreak detection

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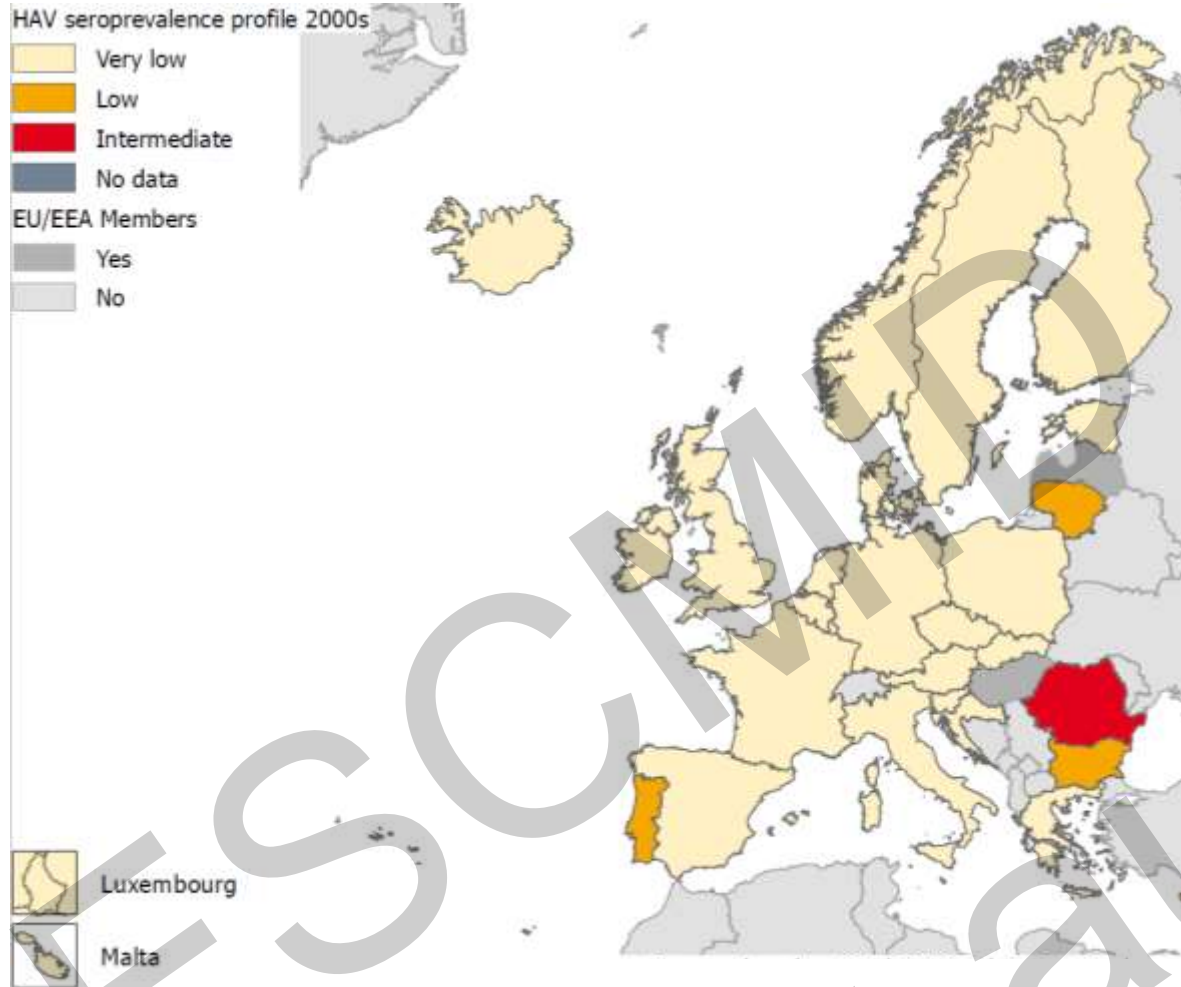
ECCMID, Amsterdam, 15 April 2019

Hepatitis A virus (HAV) infection

- Acute, self-limiting, conferring life long immunity
- Often asymptomatic (or mild) in children ≤ 5 years
- When symptomatic:
 - incubation period ≈ 30 days (2 to 6 weeks)
 - clinical expression and severity increase with age
 - case-fatality ratio: 0.1% generally, 1.8% in patients > 50 years
- Highly transmissible; mainly faecal-oral or sexual transmission (MSM*)
 - prone to person-to-person and foodborne transmission outbreaks

***MSM**: men who have sex with men

HAV endemicity (≤ 30 yrs) and groups at risk, EU/EEA 2000-2014



Carrillo-Santistevé *et al*, Lancet ID 2017

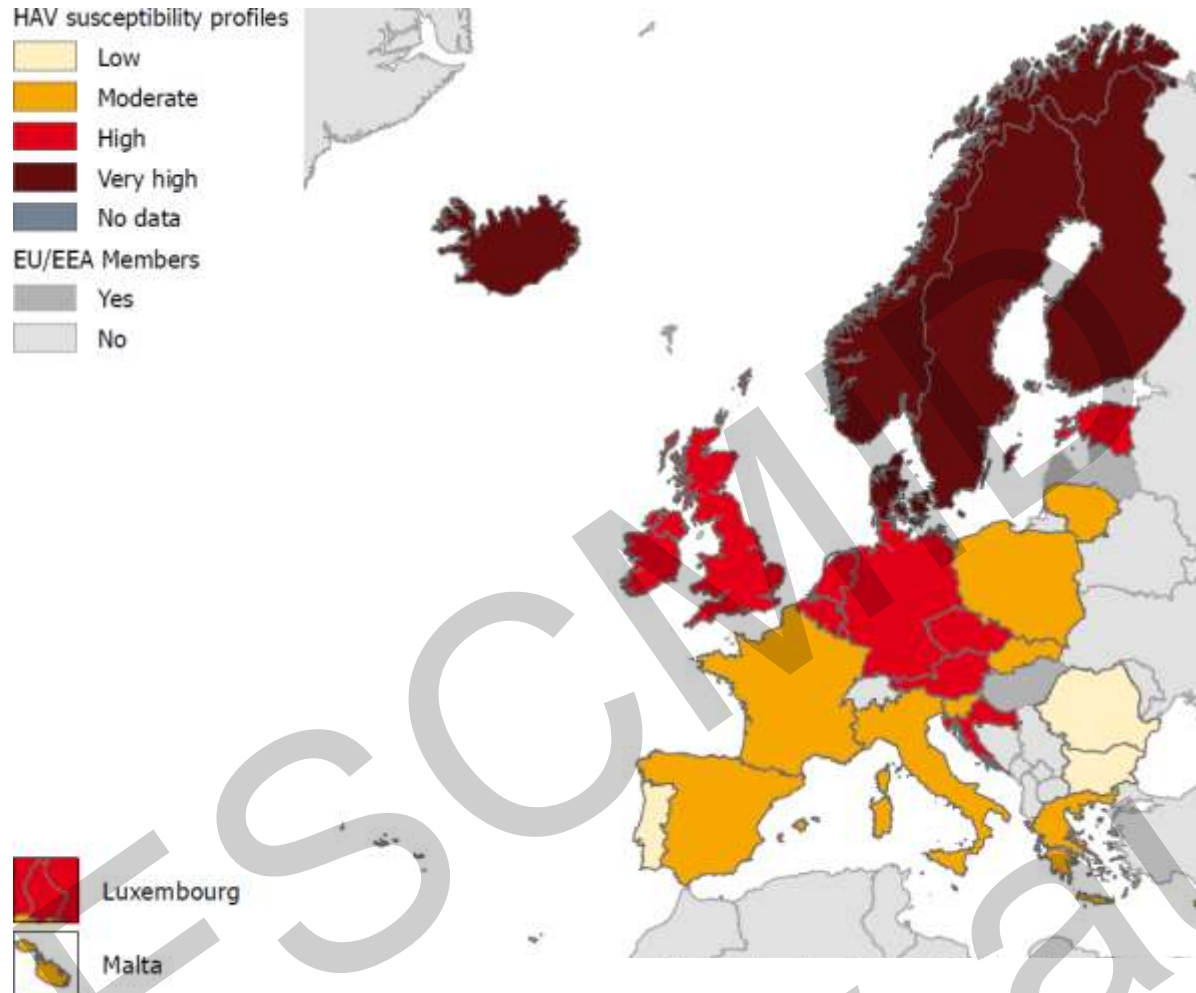
Most EU/EEA countries have low or very low HAV endemicity

Groups at increased risk of infection:

- travellers to endemic countries
- household and sexual contacts of acute cases (including MSM)
- PWID*
- users of day-care centres
- second generation migrants visiting country of origin

***PWID**: people who inject drugs

HAV susceptibility (50yrs), EU/EEA 2000-2014



High variability in HAV susceptibility in the EU/EEA

Groups at increased risk of a serious outcome:

- >50 years of age
- HCV, HBV patients
- other liver disease patients
- immunocompromised

Carrillo-Santistev *et al*, Lancet ID 2017

Multi-country outbreak detection in the EU (2013-2019)



- Outbreaks and clusters of interest detected at national level
- Countries post alerts in EPIS-FWD (or EWRS*) with outbreak sequence
- Other countries report possible matches

- Hepatitis A events reported in EPIS-FWD:

Year	Hep A events	Multi-country
2013	4	3
2014	3	1
2015	3	1
2016	3	2
2017	5	1
2018	9	4
2019	2	0

ECDC Extranet | **EPIS FWD** Epidemic Intelligence Information System Food- and Waterborne Diseases and Zoonoses

Home | News | Molecular Typing Cluster Investigations | Urgent Inquiries

*EWRS: Early Warning and Response System

Large multi-country outbreaks in the EU (2013-2018)



- Disproportionally affecting MSM (2016-2018)¹
 - as of 7 Sept 2018, 4 475 sequenced-confirmed cases in 22 EU/EEA countries
 - >15 000 cases estimated to be part of this outbreak
- Mixed frozen berries (2013-2014)²
 - 1 589 sequenced-confirmed cases in 13 EU/EEA countries
- Non-immune travellers to destinations out of the EU
 - recurrent outbreaks: Morocco (2018)³, Egypt (2013)⁴
- Viral RNA sequencing essential in outbreak detection and monitoring

¹ECDC epidemiological update 12 Sept 2018

²Severi *et al*, Eurosurveillance 2015

³Gassowski *et al*, Eurosurveillance 2018

⁴Sane *et al*, Eurosurveillance 2015

Hepatitis A virus



- HAV genome: linear positive single-stranded RNA
 - single open reading frame divided into three functional regions P1-P3:
 - P1 encodes the capsid polypeptides (virion protein VP1-VP4)
 - P2 and P3 encode the non-structural polyproteins 2A-2C and 3A-3D for virus replication
- Low mutation rate and low variability
- One single serotype
- Six HAV genotypes differing by 15–25 % in the VP1/2A region
- Genotypes further divided into subtypes A & B (7.0–7.5% difference)
- Genotype I, II and III are infectious for humans

HAVNet and HAV genotype distribution (RIVM, 2000-2017)



- Network of virologists, database and tools
- 14 EU/EEA participating countries
- Sequences + background data
- Online analysis and visualisation (BLAST), phylogeny and geographical analysis tool
- Useful to pinpoint geographical origin of sources in outbreak investigations
- **Sequencing protocol** (instructions on RNA extraction, PCR settings, primers for amplification of the VP1/2A region, reagents)

HAV genotype	HAVNet	
	n	%
I.A	1,339	55.9
I.B	780	32.6
II	4	0.2
III.A	271	11.3
III.B	0	0.0
Total	2,394	100

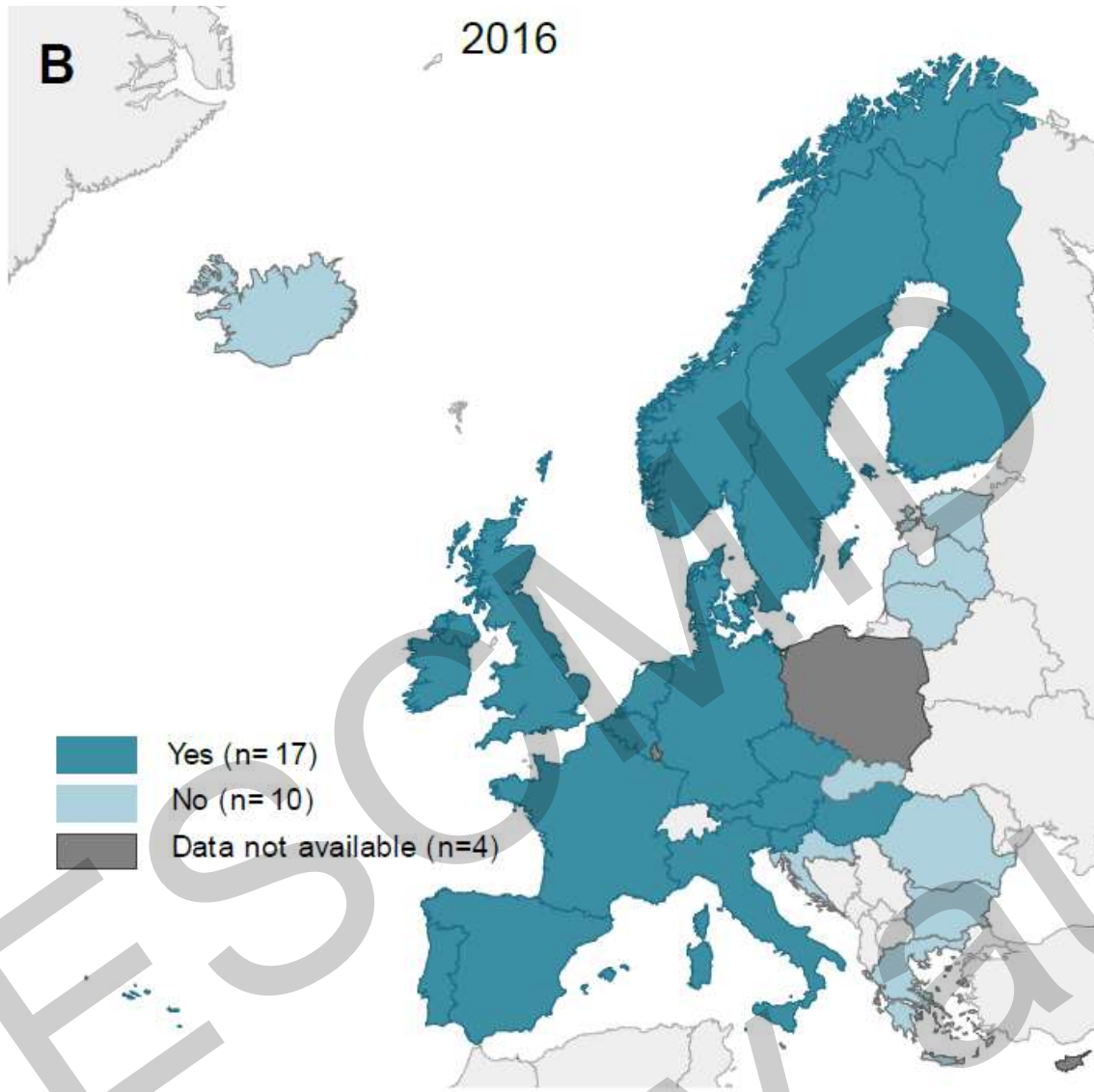
Kroneman *et al*, Eurosurveillance 2018
(database extracted on 27 February 2017)

Harmonisation of HAV sequencing methods in the EU/EEA, ECDC 2013-2018



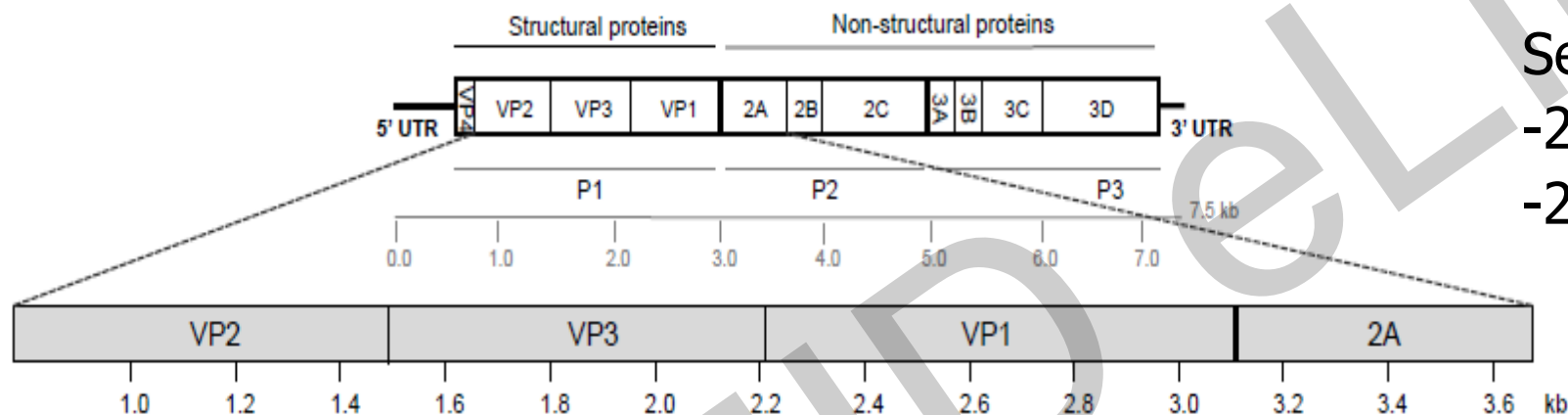
- ECDC efforts towards harmonised procedures for cross-border outbreak detection and investigation:
 - same amplified target region for sequencing and phylogenetic analysis
 - length of the target regionAND improved
 - exchange of HAV sequence information between countries
 - collaborations amongst EU/EEA countries
 - collaborations between human and food sectors
- 2014 & 2016, two ECDC surveys on sequencing capacities in EU/EEA

HAV sequencing in the EU/EEA, 2016

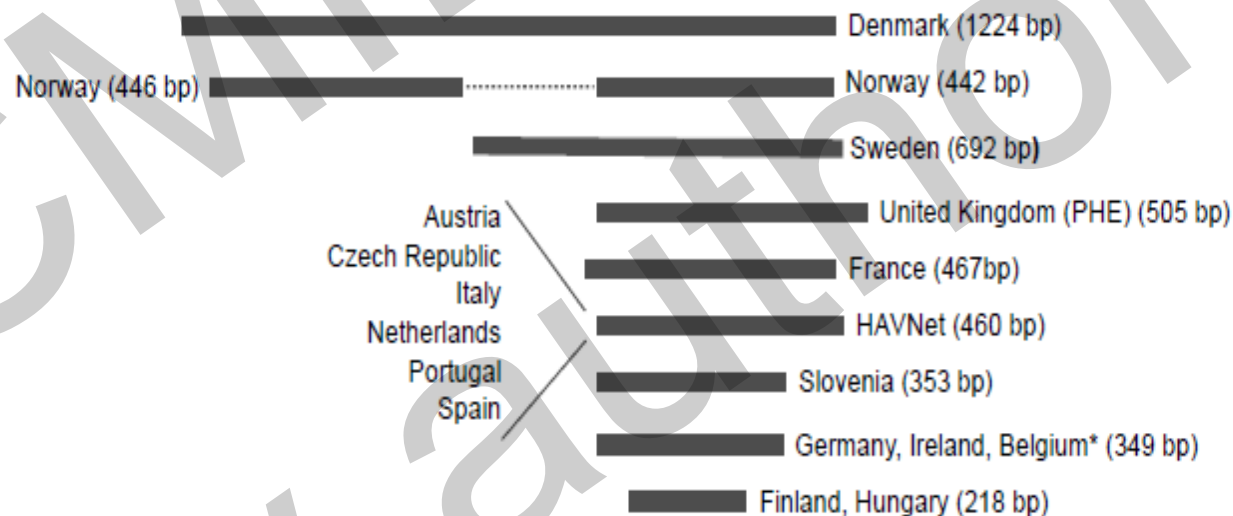


Sequencing performed in:
-2014; 15/23 countries
-2016; 17/27 countries

HAV genome and amplification targets for partial genome sequencing (EU/EEA, 2016)

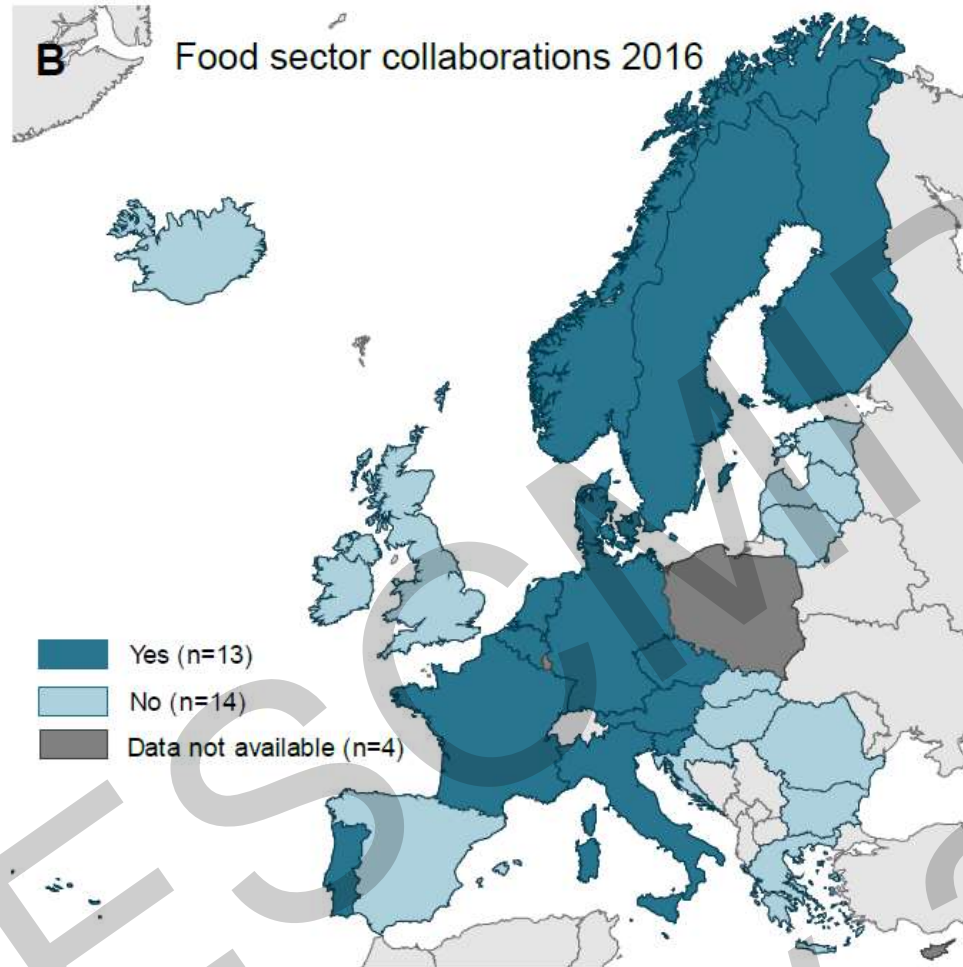


Sequencing of the VP1/2A junction:
 -2014; 15/15 countries
 -2016; 17/17 countries



2016

Collaborations between human and food sectors (EU/EEA, 2016)



Collaborations with the food sector for
sequence comparison:
-2014; 10/23 countries
-2016; 13/27 countries

ECDC strategic framework on integration of genomic typing in multi-country outbreak investigations (2019-2021)



- Objective for HAV sequencing is to support multi-country outbreak investigations
 - verification of multi-country outbreaks
 - support investigations of outbreak sources/vehicles jointly with EFSA
 - assessment of the effectiveness of control measures
- ECDC will collaborate with HAVNet to promote HAV sequencing using the HAVNet protocol (sequencing of the VP1/2A region) or WGS
- Explore added value & feasibility of HAV sequencing-based surveillance, but postpone EU-wide implementation until further review in 2021

Whole genome sequencing for HAV

- WGS is useful for specific outbreak investigations
 - improves source tracing
 - in large outbreaks, allows identification and investigation of sub-clusters
- Challenges:
 - requires samples with high viral load
 - expensive
- No routine implementation yet for (inter)national surveillance and outbreak detection

Conclusions

- Partial genome sequencing value:
 - outbreak detection, investigation & monitoring
 - distinguish sporadic cases from outbreak cases
 - identification of vehicle of infection (foodborne outbreaks) and/or source tracing
- To make best use of it:
 - target the same genomic region (HAVNet protocol)
 - perform timely sequencing, analysis and multi-country comparison
 - collect epidemiological information from cases
 - compare with food sector
- Whole genome sequencing added value:
 - improve source tracing and investigation (person-to-person outbreak)

Transparency Declaration

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ESCMID eLibrarian
by author

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**Thanks for
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