



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

PROTOCOL

Molecular detection and typing of
VP1-2A region of Hepatitis A virus (HAV)



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Acknowledgements

- Harry Vennema, National Institute for Public Health and the Environment (RIVM) harry.vennema@rivm.nl
- Annelies Kroneman (RIVM-IDS)
- Annemieke Mulder, Agnetha Hofhuis, Eelco Franz (RIVM-EPI)
- Cornelia Adlhoch (ECDC)
- Rita de Sousa, Instituto Nacional de Saúde, Lisbon, Portugal
- HEVnet members (hevnet@rivm.nl)
- HAVnet members (havnet@rivm.nl)
- Koen Deforche (Emweb)



Comparison of HAV and HEV: similarities

	HAV	HEV
Route of infection	Primarily oral Blood or blood products	
Incubation period	3-5 weeks	
Diagnosis acute infection	High IgM titer	
Pathogenesis	Ingestion, infection of liver, secretion in feces	
Gender distribution	60% men	
Virology	Small +stranded RNA virus Single serotype, multiple genotypes	
Genetic diversity	Many different strains within genotypes	

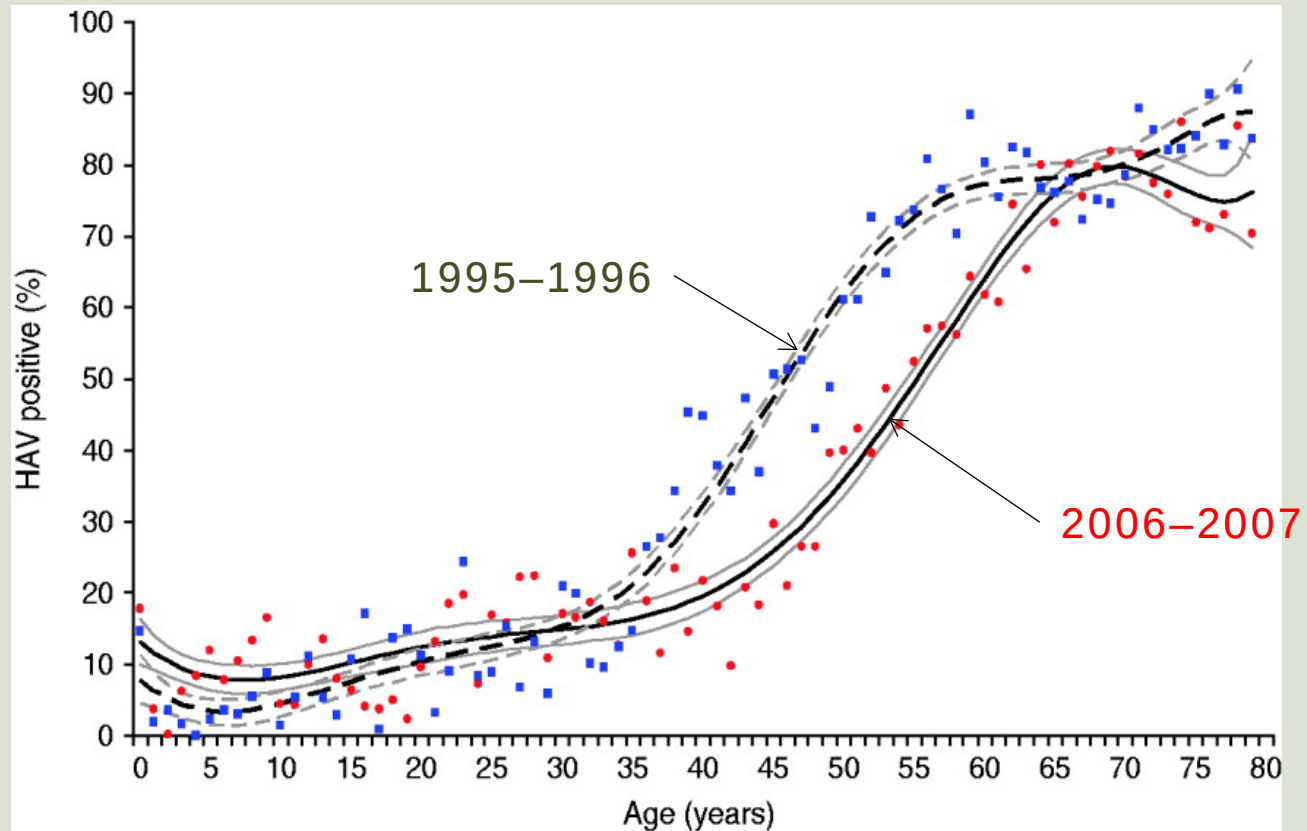


Comparison of HAV and HEV: differences

	HAV	HEV
Person-to-person transmission	Efficient	Inefficient
Disease in adults	>90%	<10%
Prevalence/incidence	Low	High
Point-source outbreaks	Frequent	Extremely rare
Food source	Exotic	Common
Vaccine	Available	Not available
Age distribution patients	Young	Elderly
Duration of immunity	Lifelong	Not known
Origin of infections	Imported	Endemic
Genetic variability	Low	High



Prevalence of hepatitis A IgG



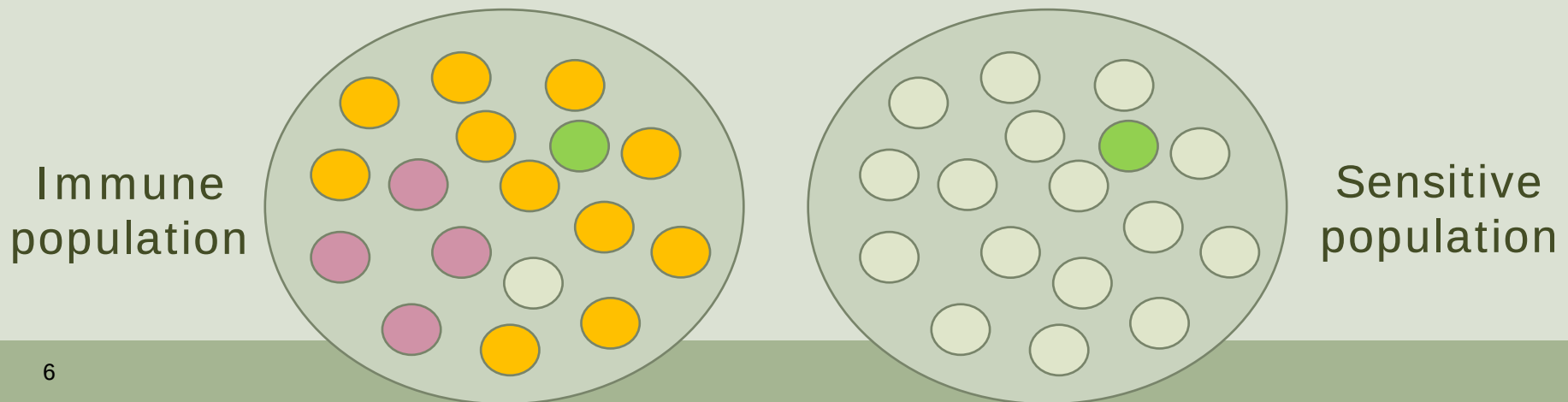
Changing risk profile of hepatitis A in The Netherlands: a comparison of seroprevalence in 1995-1996 and 2006-2007.

Verhoef L1, Boot HJ, Koopmans M, Mollema L, Van Der Klis F, Reimerink J, Van Pelt W. Epidemiol Infect. 2011 Aug;139(8):1172-80



Objectives HAVNet

- Building a reference database capturing the worldwide genetic diversity of HAV, in order to be able to annotate new sequences for geographical origin or risk group
- To identify geographically dispersed outbreaks
- To support epidemiological outbreak investigations and source tracing
- To supply a standard typing protocol to new and participating laboratories





HAV in blood donations

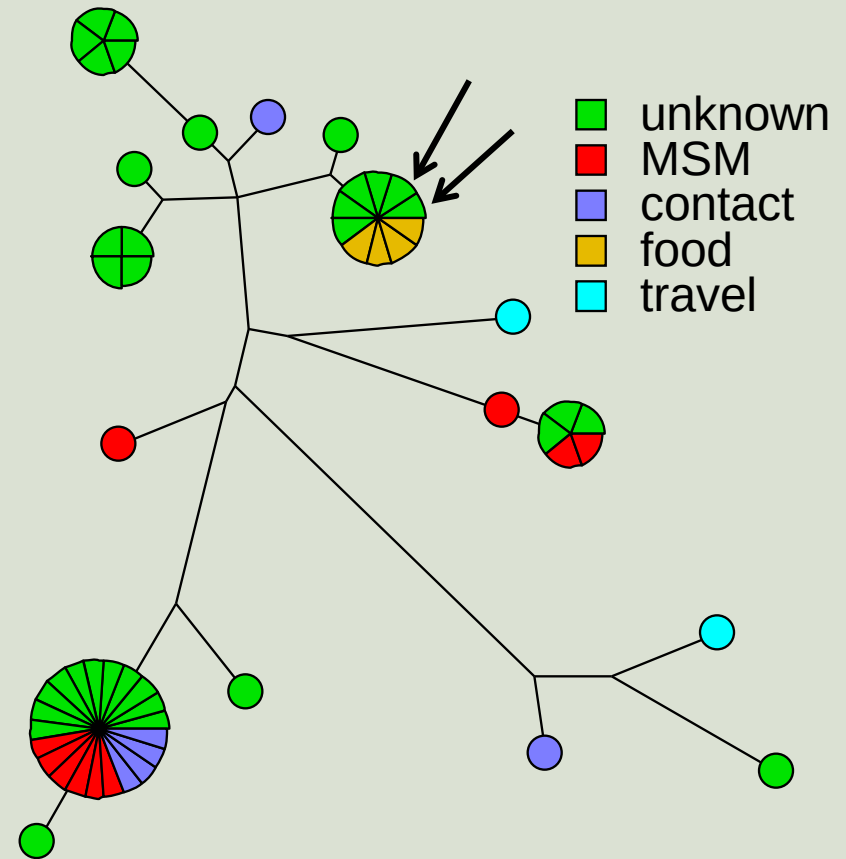
- Large outbreak of Hepatitis A in Europe in 2017
- Twice the number of cases as compared to previous years in Europe
- Individual countries 2 to 40-fold more cases
- Primarily men-who-have-sex-with-men (MSM)
- Spill-over to general population
- HAV NAT screening to avoid Transfusion-Transmitted Hepatitis A



HAV NAT screening of donations

Netherlands: 2 positive donations January 2018

- 2 female donors became symptomatic 3 weeks later
- No known risk factor
- Typing of the strains
- Both part of small cluster of identical strains
- Clustered in time, different regions, attributed to imported berries
- Thanks to Boris Hogema (Sanquin) for sharing
- 2018: 46 of 55 cases part of cluster





Objectives HEVnet

- Trends of human HEV
 - Circulating subtypes in time and place
- Molecular surveillance
 - Distribution of subtypes in people and animals
- Cluster and outbreak detection
 - Sequence comparison from different sources
- Source tracing/attribution
 - Which reservoirs contain the same sequences as patients
- Population genetics
 - Spread and evolution of hepatitis E virus
- Pathogenicity of strains
 - Association between subtypes and severity of disease



Requirements

- Minimum required information:
 - Human cases
 - Time & place
 - (sub)type according to a standardized nomenclature
- Standardized metadata:
 - Demographic & epidemiological
 - Clinical data
- Sequences of an agreed genomic region
- Animal/food/environmental samples
- Timely reporting



Experts involved

- Public health epidemiologists
 - Trends of human HEV
 - Cluster and outbreak detection
 - Source tracing, attribution
- Clinical virologists
 - Pathogenicity of strains
- Food, environmental, veterinary virologists
 - Source tracing, attribution
 - Cluster and outbreak detection
- Physicians
 - Cluster and outbreak detection
- Molecular virologist
 - Molecular surveillance, population genetics



Comparison of HAVnet and HEVnet

	HAVnet	HEVnet
Since	2010	2017
Sequence data	All available, incl. Genbank	Recent and reference
Sequence region	VP1/2A	Part of ORF2
Metadata	Travel origin often unknown	Source usually unknown
Sample origin	Human, food	Human, animal, food, environment
Typing protocol	Available	Available
Typing/subtyping tool	Online	Online
Expected results	Outbreak detection	Trend analysis
Success	Several examples	High expectations



Typing according to a standardized nomenclature

FileEditViewHistoryBookmarksToolsHelp

Hepatitis E Virus Genotyping Tool

←→↺🏠

🔒 RIVM (Rijksinstituut voor Volk... (NL)

https://www.rivm.nl/mpf/typingtool/hev/

⋮📌☆

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Hepatitis E Virus Genotyping Tool Version 0.0

Submit JobMonitor job [37271558]How to citeIntroductionHow to useExample sequences

Hepatitis E Virus Genotyping Tool Results

You may bookmark this page to revisit results of this job (37271558) later.

Download results: [XML File](#) [Table \(Excel format\)](#) [Table \(CSV format\)](#) [Sequences \(Fasta format\)](#)

Name	Length	BLAST result	BLAST score	phylo major result	phylo major support	phylo minor result	phylo minor support	Report	genome
AB222087 HepE3	821	Hepeviridae Orthohepevirus A	92.69184	HEV3	99.0	HEV3a	93.0	Report	<div><div></div><div>15' end2.5k5k7.5k3' end</div></div>

Developed by: [RIVM](#) (Harry Vennema, Annelies Kroneman) and [Emweb bvba](#).

Contact: TBD

Hepatitis E Molecular Platform

Logged in as **Harry Vennema** Collaborator National Institute for Public Health and the Environment [\(Logout\)](#)

- Cases
 - List
 - Add
- Batch Import
- Query
- My Account

Case List

[Reset All](#) [Show filter](#)

Case Nr	Submission Date	Country (submitter)	Sample from	Genotype	Subtype
TLS-170032732	21-03-2018	France	Human	HEV3	Could not assign
TLS-162092335	21-03-2018	France	Human	HEV3	HEV3c
TLS-170032721	21-03-2018	France	Human	HEV3	HEV3f
TLS-160702135	21-03-2018	France	Human	HEV3	HEV3c
TLS-163552177	21-03-2018	France	Human	HEV3	HEV3f
TLS-170032734	21-03-2018	France	Human	HEV3	HEV3f
TLS-170032719	21-03-2018	France	Human	HEV3	HEV3c
food_CH_BLV00916_B1	16-03-2018	Switzerland	Food	HEV3	HEV3s (p)
18-HE-00586	15-03-2018	Belgium	Human	HEV3	HEV3f
IT17NA2016	06-03-2018	Italy	Animal	HEV3	HEV3i
IT75BO2012	06-03-2018	Italy	Animal	HEV3	HEV3o (p)
18-HE-00476	05-03-2018	Belgium	Human	HEV3	HEV3c
18-HE-00449	05-03-2018	Belgium	Human	HEV3	HEV3c
18-HE-00435	05-03-2018	Belgium	Human	HEV3	HEV3l (p)
17-HE-01519	05-03-2018	Belgium	Human	HEV3	Could not assign
17-HE-01036	05-03-2018	Belgium	Human	HEV3	HEV3c



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Hepatitis E Molecular Platform

Logged in as **Harry Vennema**

Collaborator

National Institute for Public Health and the Environment

[\(Logout\)](#)

- > Cases
- > Batch Import
- > Query
 - > Using criteria
 - > Using Database IDs
 - > Using similarity
- > My Account

Query by properties

Criteria

AND Between

AND

Results

Count 160 Cases (160 sequences) matched by query

Download sequences (Fasta) [result.fasta](#)

Results (up to first 1000 results)

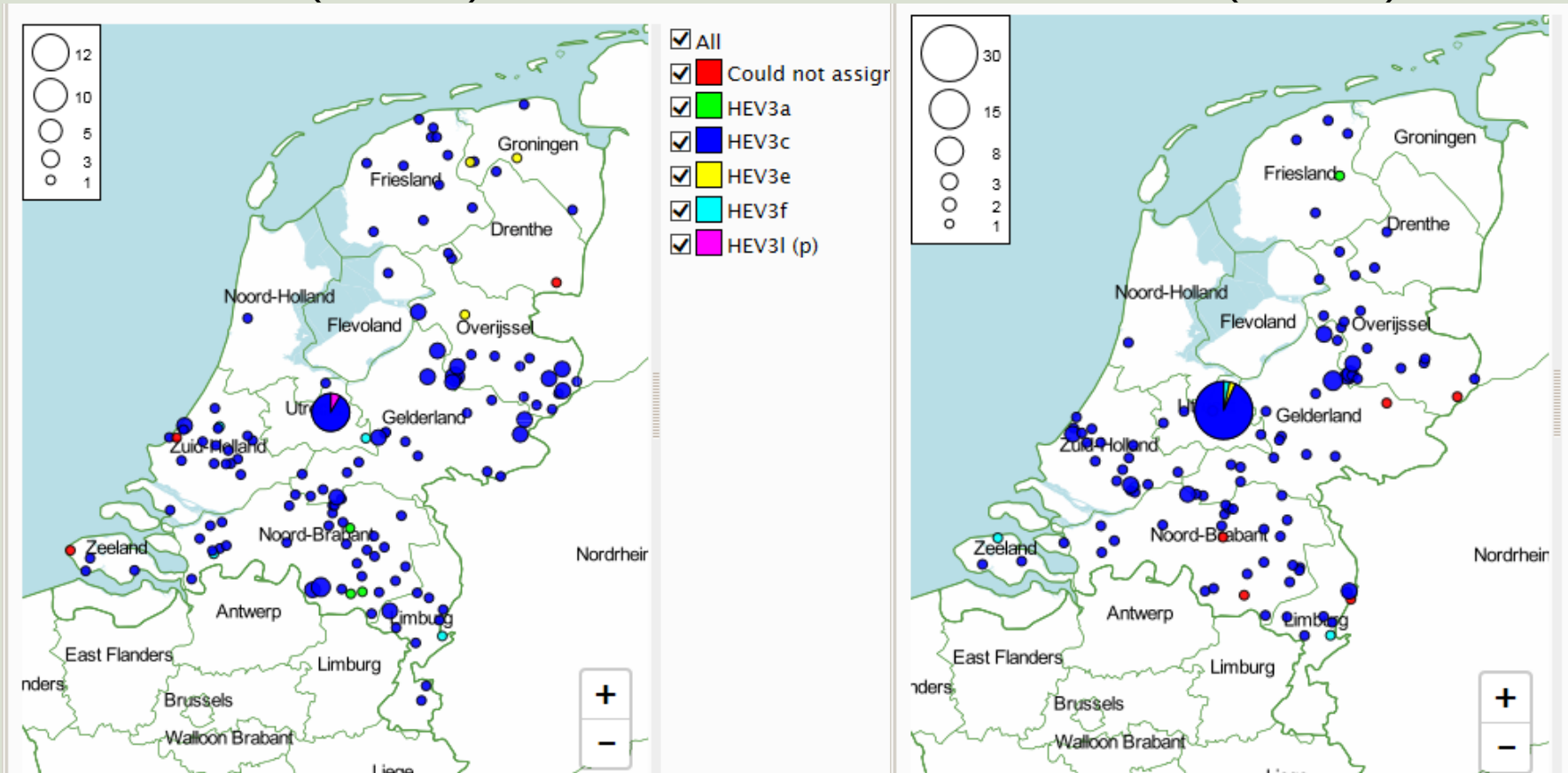
Case Nr	Submission Date	Country (submitter)	Sample from	Genotype	Subtype
NL_3001500089	15-09-2017	Netherlands		HEV3	HEV3f
NL_3001500233	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500261	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500292	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500293	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500294	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500354	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500395	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500399	15-09-2017	Netherlands		HEV3	HEV3c
NL_3001500450	15-09-2017	Netherlands		HEV3	HEV3c



Regional distribution of HEV cases

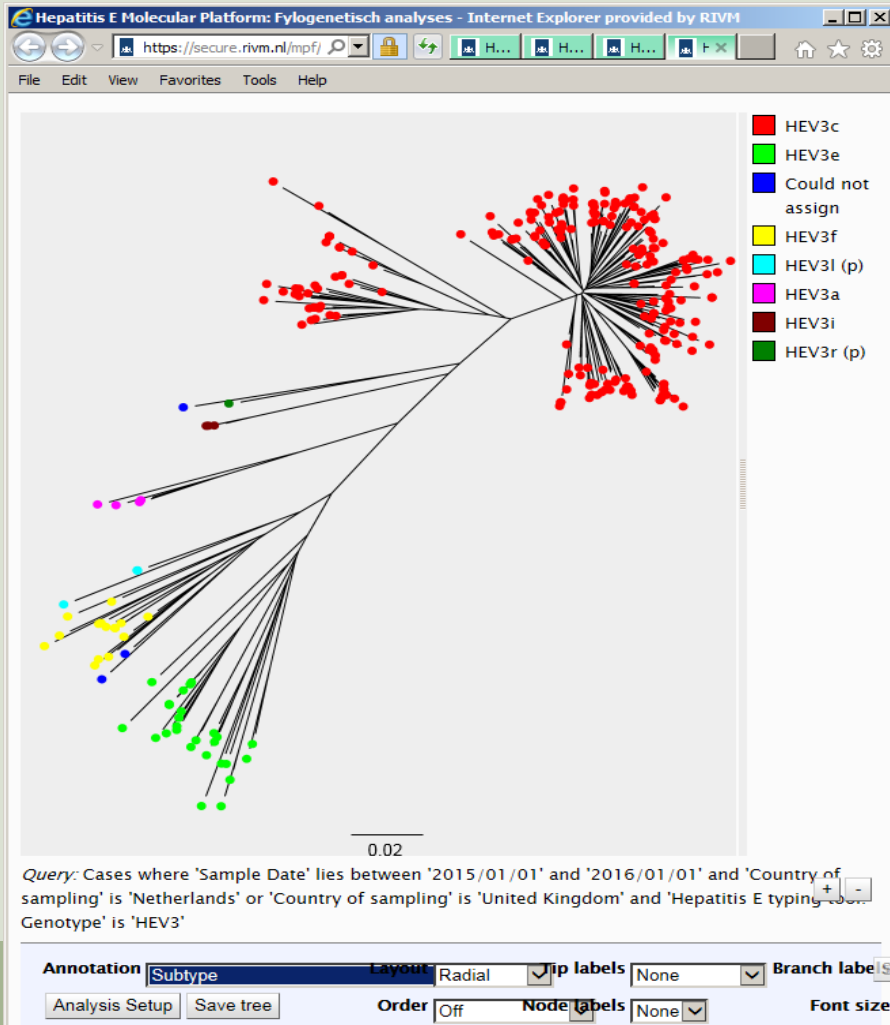
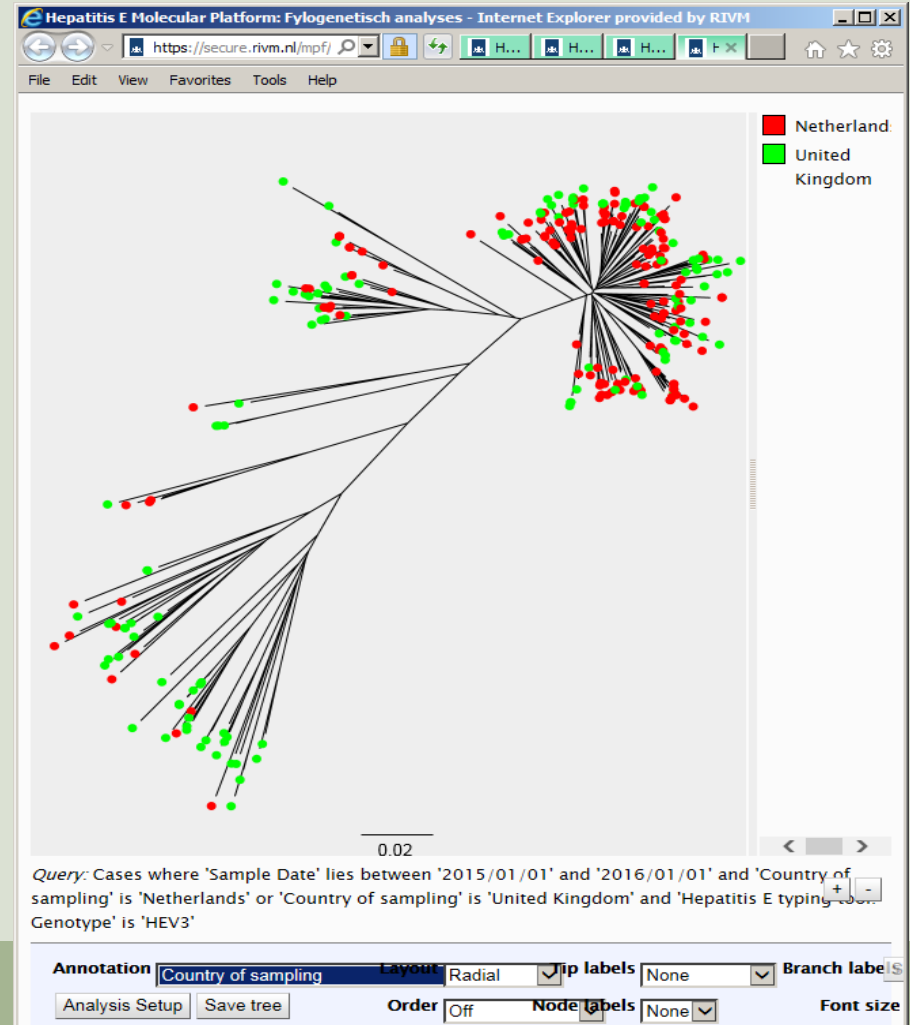
2015 (n=160)

2016 (n=141)





HEV3 subtype distribution UK, NL, 2015





Conclusions

- A network of experts with a database of shared information can help to tackle public health problems
- The database should be setup to systematically capture microbiological data combined with metadata
- A username and password protected database provides a safe place to share data in a timely manner
- HAVnet: several examples of international outbreak detection with various risk factors, timely sharing of information provides clarity in emergency situations and actionable intelligence for public health authorities
- HEVnet: almost 1000 entries already, sequences with varying amount of metadata, several prospective new subtypes identified