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

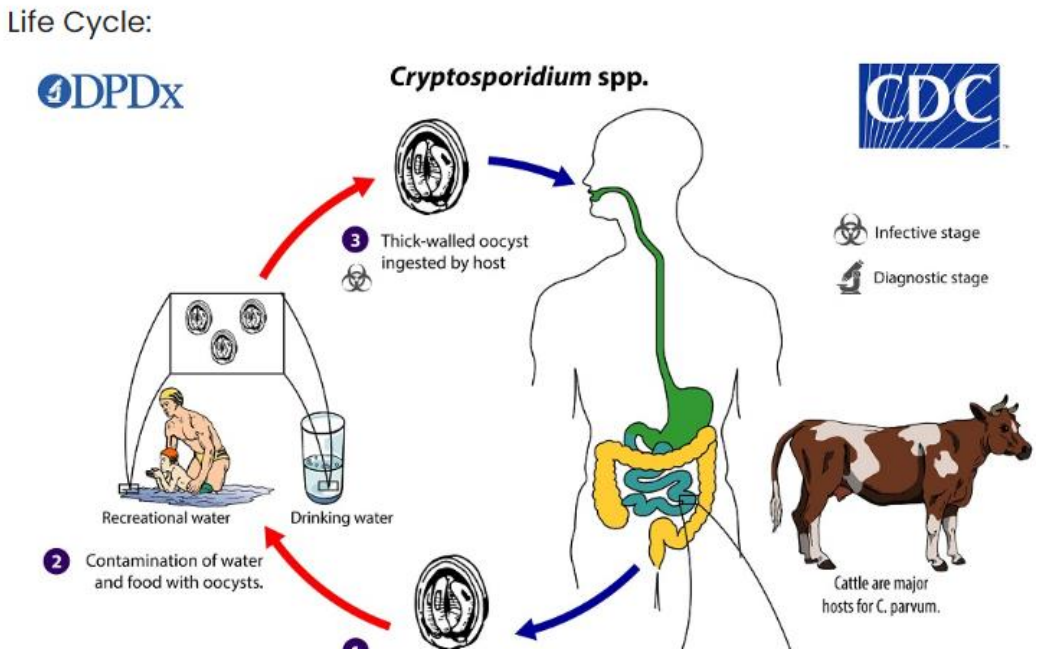
MLST and MLVA for phylogenetics and cluster detection of *Cryptosporidium parvum* of human and animal origins

Kees van der Ark

kees.van.der.ark@rivm.nl

Introduction

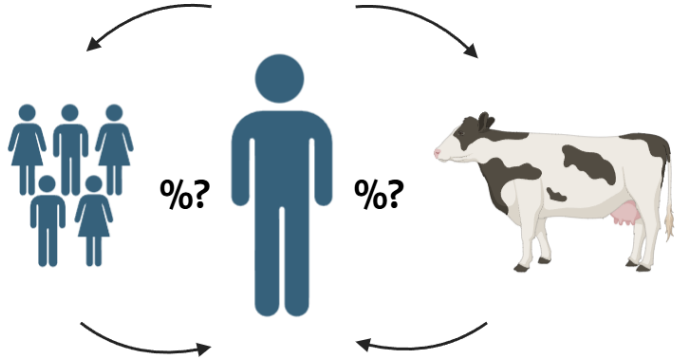
- *Cryptosporidium parvum* is zoonotic → cattle are besides human primary host
- *C. parvum* is highly infectious and associated with large outbreaks
- *C. parvum* can be obtained via direct contact with feces of human or cattle, contamination of cattle feces in agriculture, swimmingpool outbreaks
- Limited research estimating the contribution of cattle to human ***C. parvum*** infection



elise.groenen@rivm.nl

Research questions

Research aim 1: Estimate the percentage of human samples that cluster with cattle samples



Research aim 2: Compare genotyping methods for outbreak detection

Introduction – genotyping methods

Current standard genotyping method:

- gp60 → gene consisting of a region with SNPs and multiple regions with repetitive motives

Newer genotyping methods:

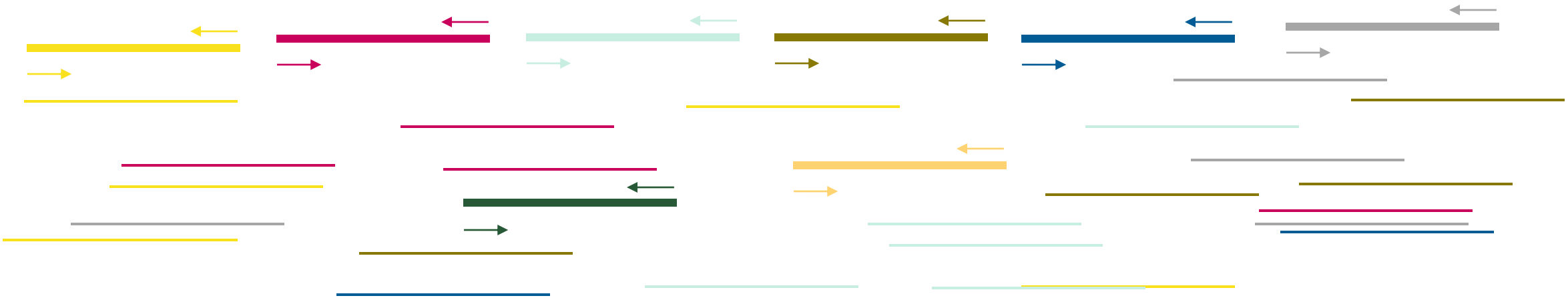
- Multiple Loci Sequence Typing (MLST) → consisting of a set of 8 genes with SNPs: suitable for phylogenetic analyses and cluster detection (Troel *et al.)*

- Multiple Loci Variable tandem repeat Analyses (MLVA) → consisting of a set of 7 genes with repetitive motives: only suitable for cluster detection (Risby *et al.*)

MLST: https://www.sciencedirect.com/science/article/pii/S2667114X25000688?ref=pdf_download&fr=RR-2&rr=a319591c0dce90cc
MLVA: Application of a new multi-locus variable number tandem repeat analysis (MLVA) scheme for the seasonal investigation of Cryptosporidium parvum cases in Wales and the northwest of England, spring 2022 - ScienceDirect

MLST

- 8 MLST genes, 1(!) PCR reaction
- Barcode and sequence 24 samples on one MinIon flowcell (equals 384 sanger reactions)

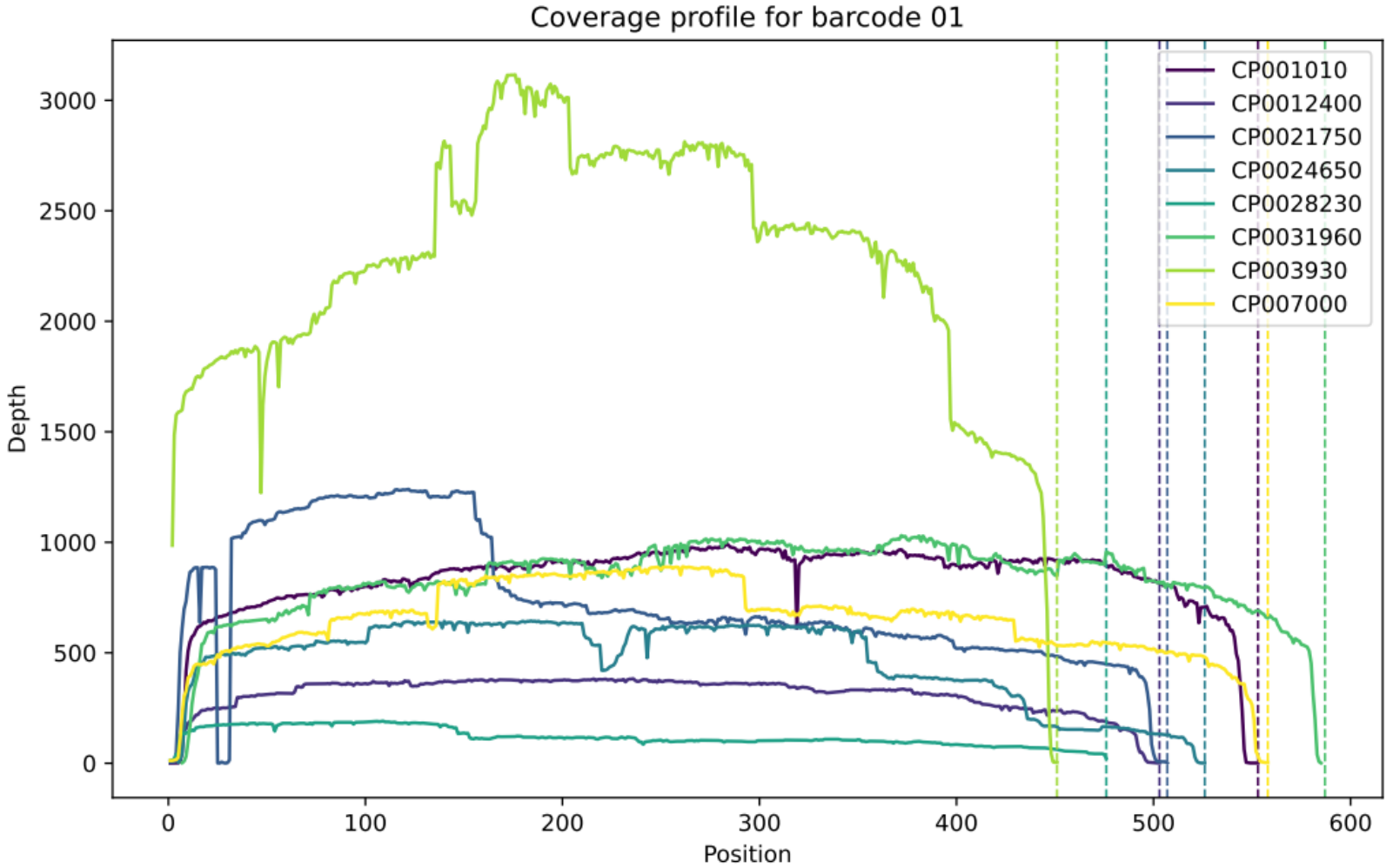


MLST

- 8 MLST genes, 1(!) PCR reaction
- Sort reads per samples using genetic barcodes
- Map reads against reference genes to determine consensus



MLST – Coverage profile per gene

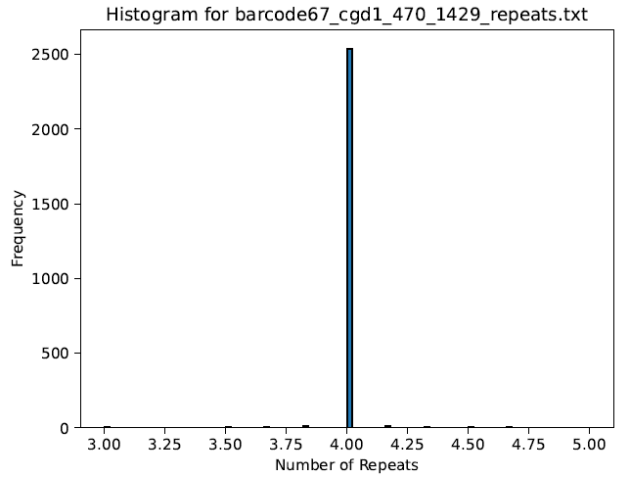


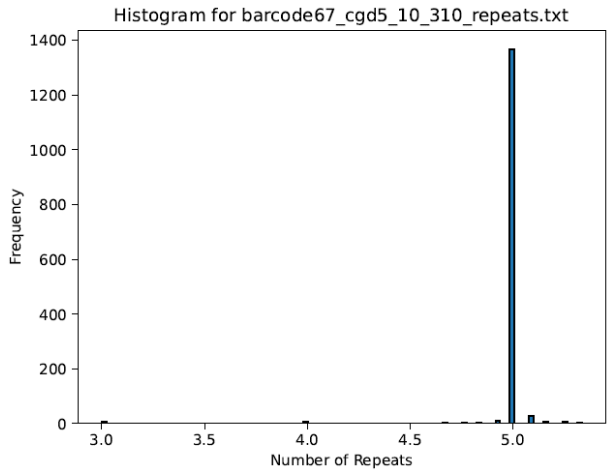
MLVA

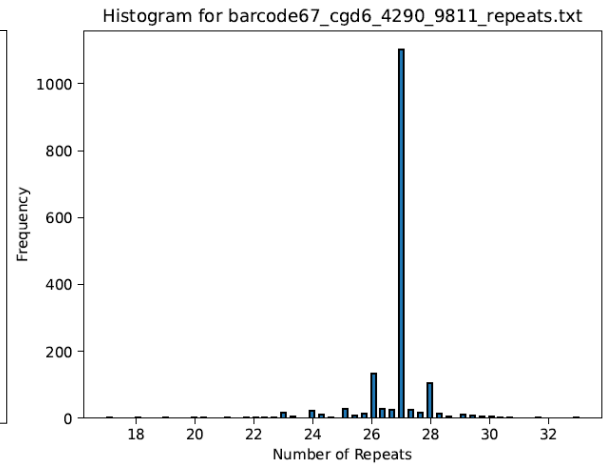
- 7 MLVA genes of varying length. 1(!) PCR reaction.
- Search all reads for primer sequences, per primer pair
- In case of a match, divide the length between the primers by the repeat length = number of repeats
- Which repeat number is most prevalent?
- MLVA code: 2-42-3-5-33-5-1

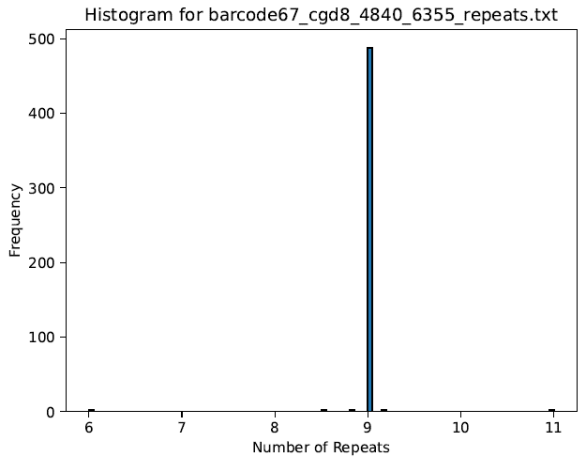


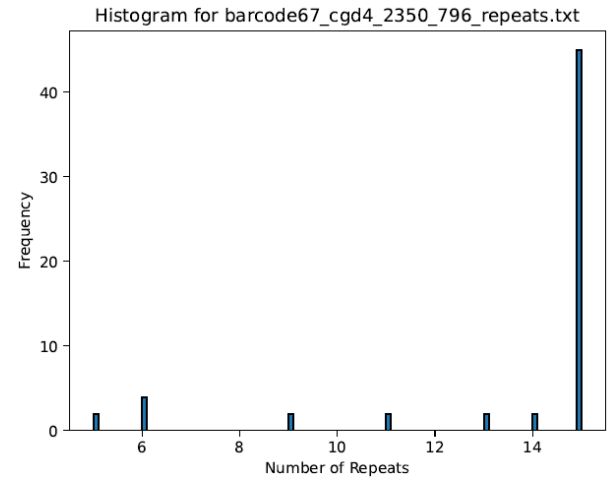
MLVA – Repeat histograms

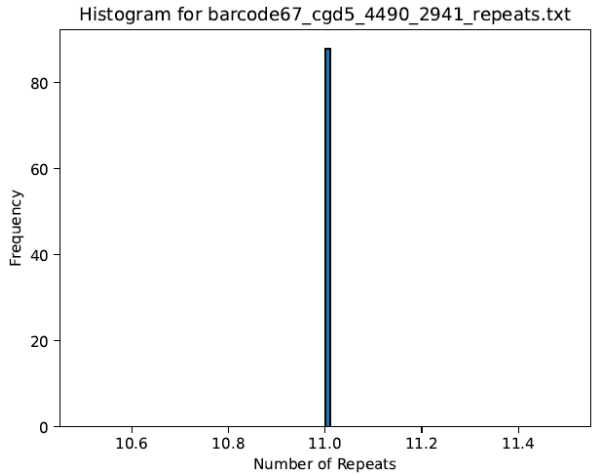


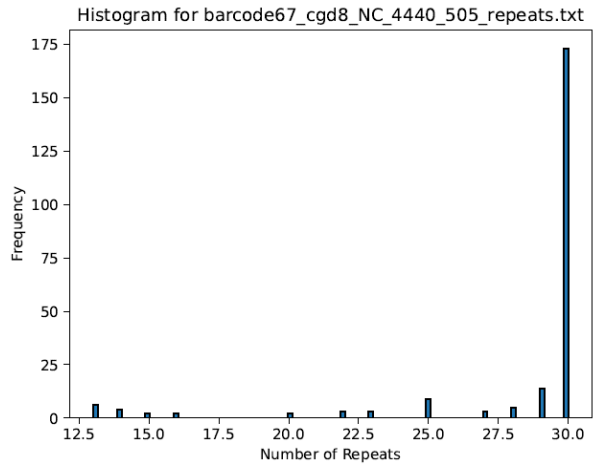








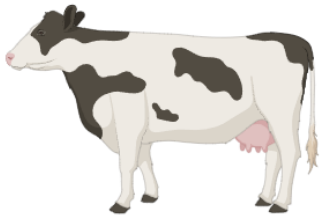




Sample set



- 91 human samples 2021 and 2022



- 71 cattle samples 2021
- Outbreak in wildlife shelter: 6 hedgehog cases + one human case in 2022



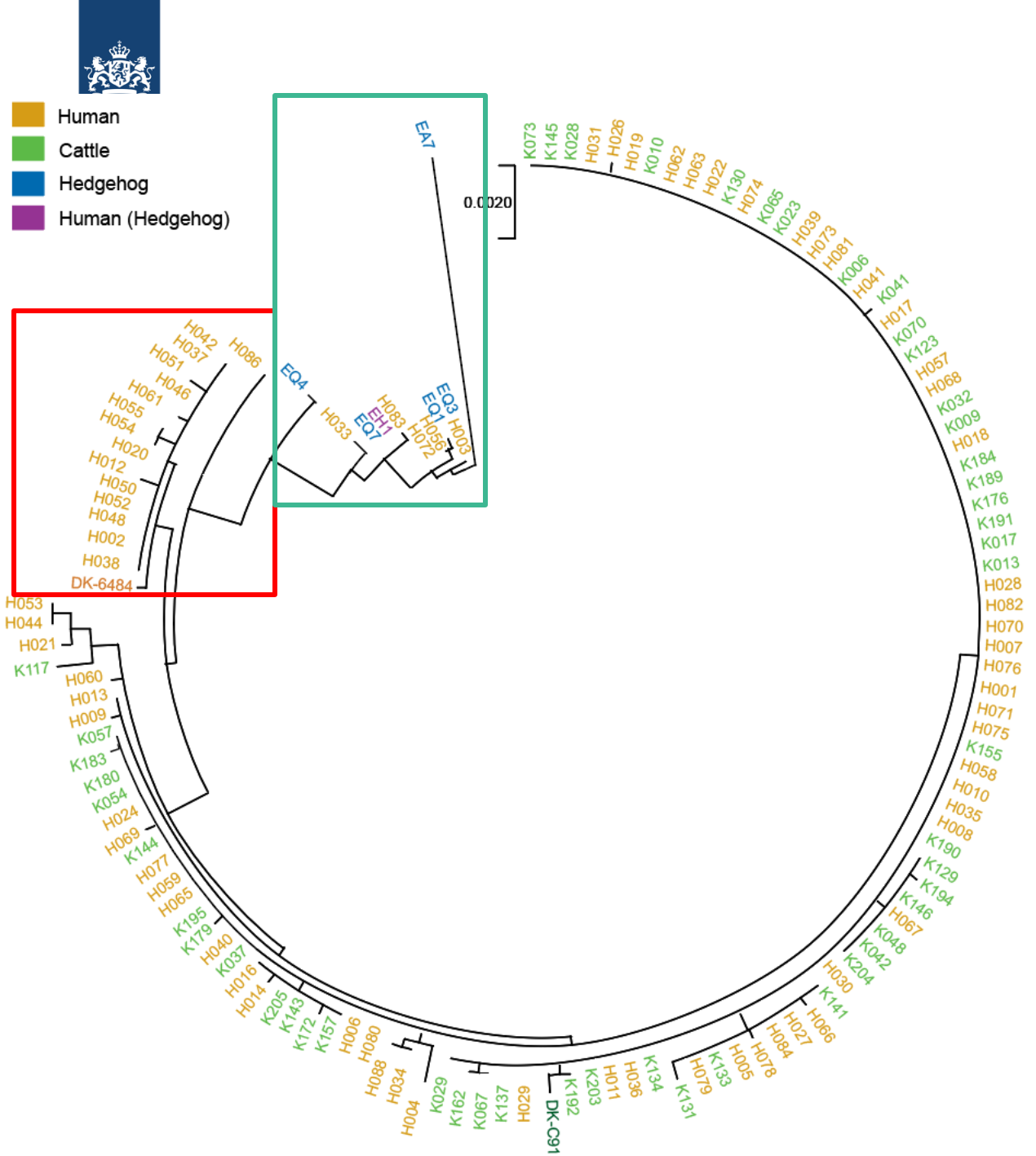
Labworkflow

Multiplex PCR + Nanopore sequencing

Research aim 1:
Estimate the percentage of human samples that cluster with cattle samples

Three general clusters:

- Human and cattle mixed
- Only human
- Human and hedgehog

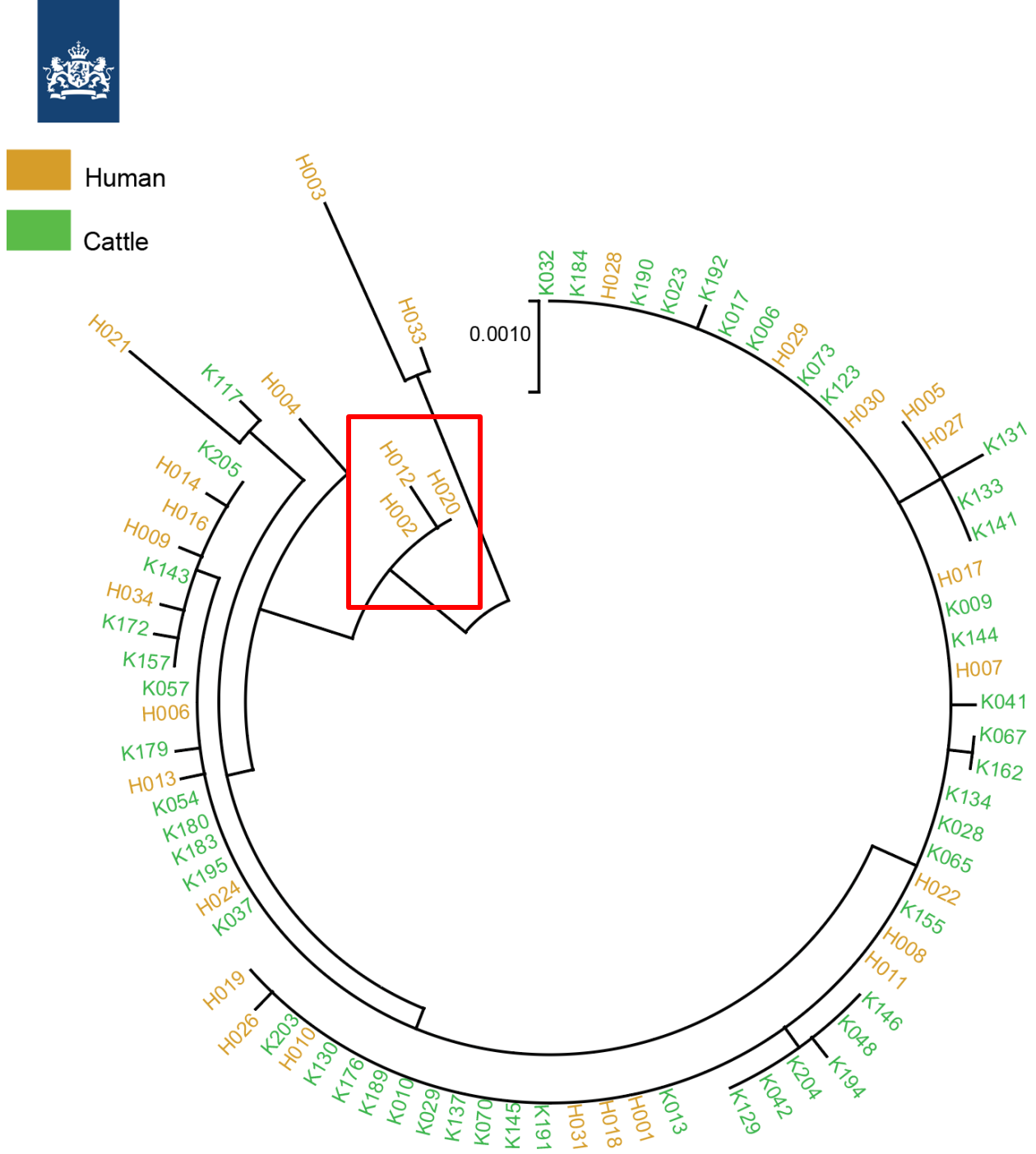


Research aim 1: Estimate the percentage of human samples that cluster with cattle samples

Only human and cattle samples from 2021 → 30 human and 50 cattle

- 17 out of 30 (56.7%) human samples clusters directly with cattle samples
- 2 out of 30 (6.7%) human samples clusters with only human samples

Expert elicitation estimated 27% human transmission and 13% animal (Havelaar *et al.*)



Conclusions - research aim 1:

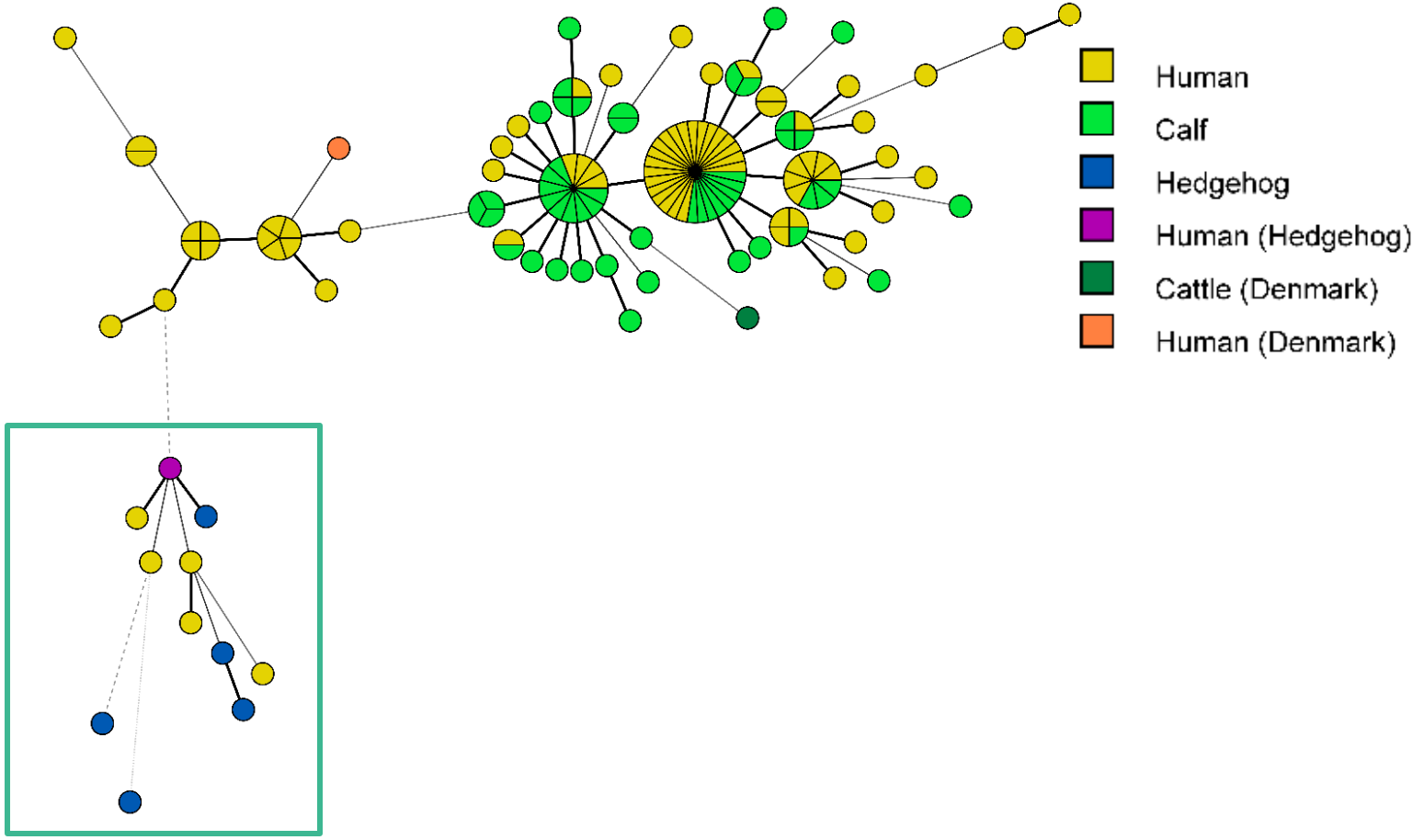
- Approx. half of human samples contain the same or highly similar MLST type to cattle (56.7%)
- MLST suggests that cattle play an important role in human *C. parvum* infection
- Still unclear which transmission route contributes most to human *C. parvum* infection (direct contact with feces, contaminated food or water etc.) → research that links genotypes with transmission pathways via questionnaires

Research aim 2: Compare genotyping methods for outbreak detection

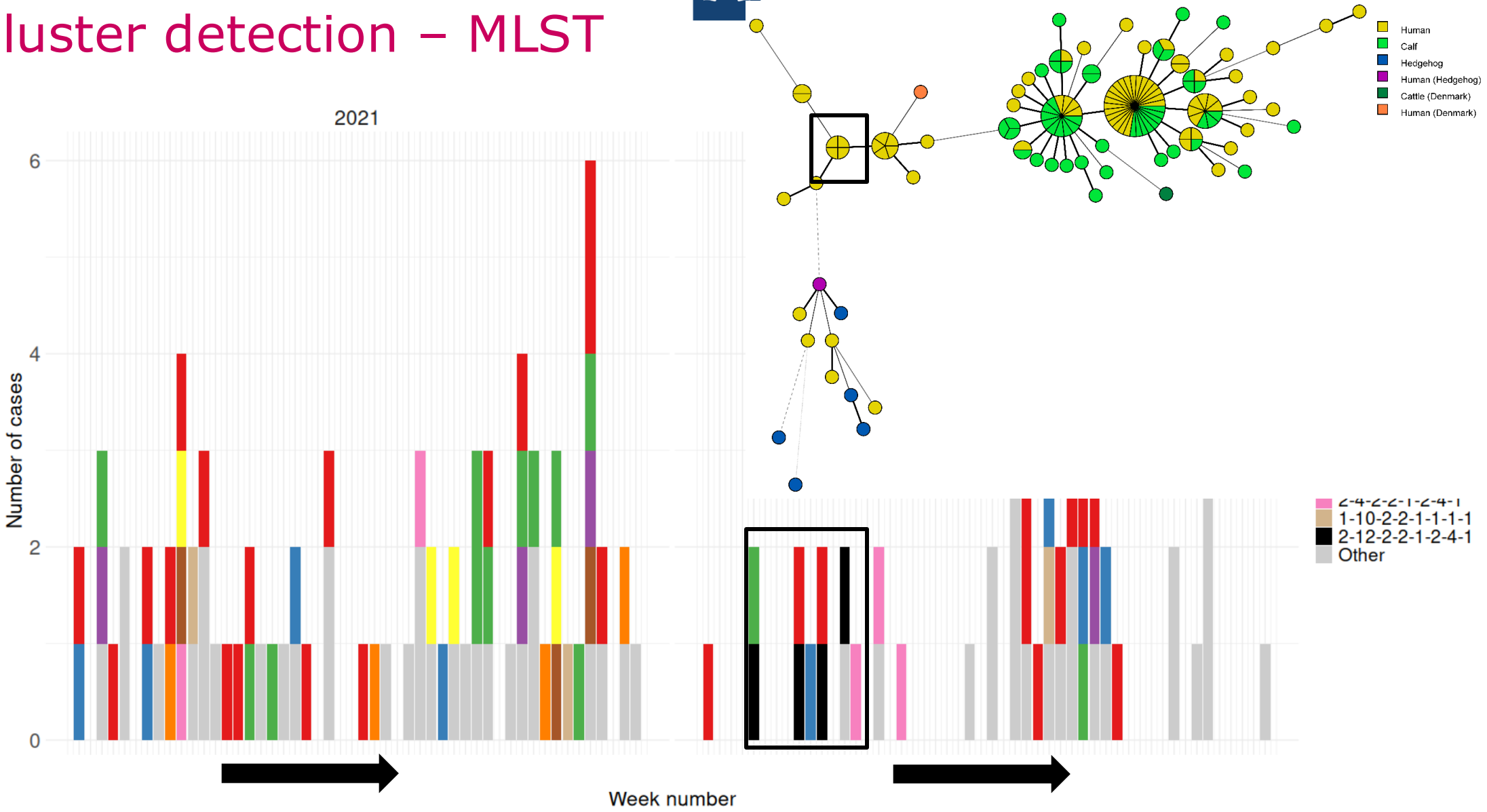
Cluster detection – MLST

DNA sequences are converted to allelic profiles (e.g. 1-4-1-1-2-3-2-10)

Minimum spanning tree to measure distance between samples



Cluster detection – MLST

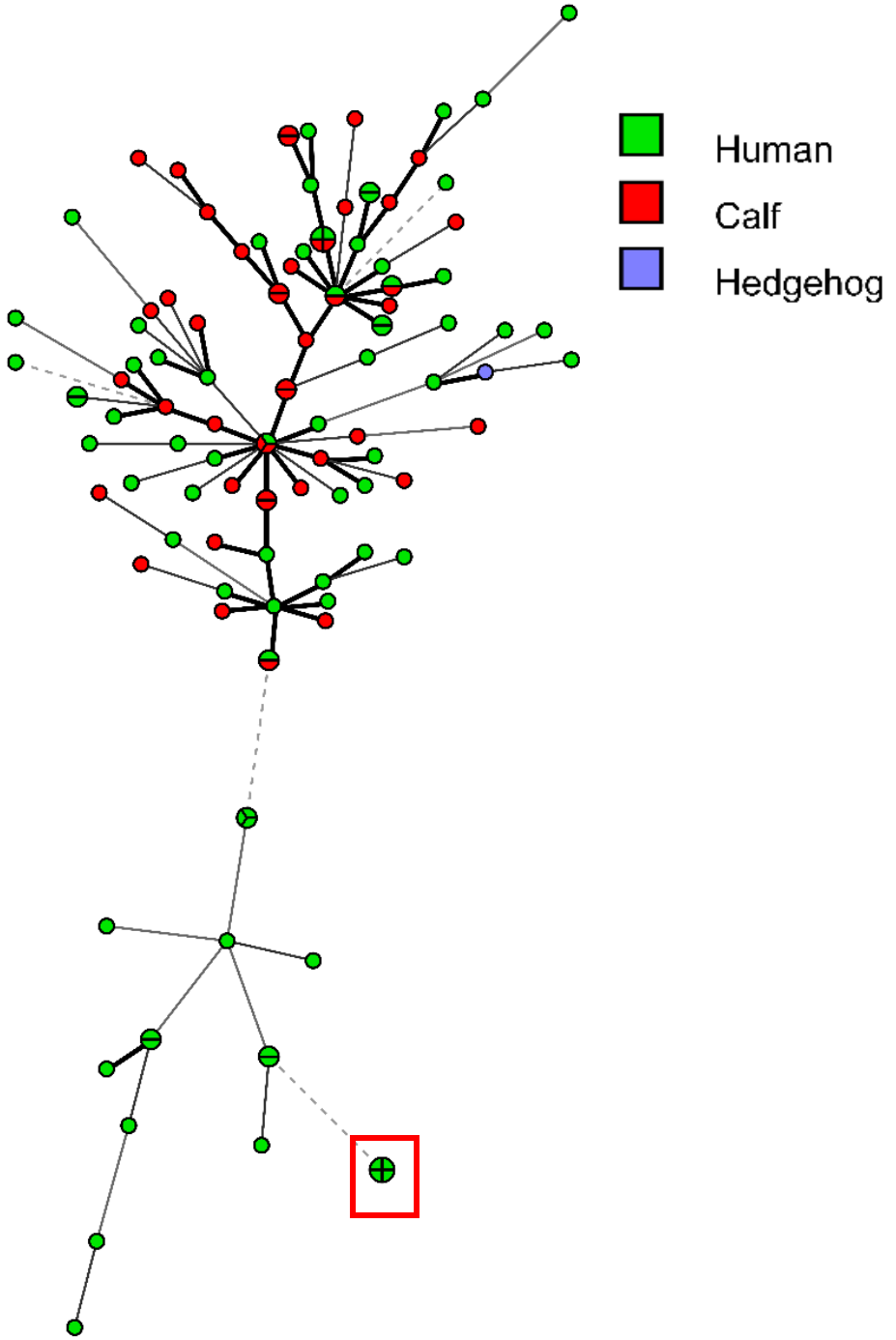


Similar genotype measured in 8-week span

Cluster detection – MLVA

Counting of repeat units results in MLVA profiles (e.g. 1-4-2-3-2-16-2)

Only one hedgehog sample

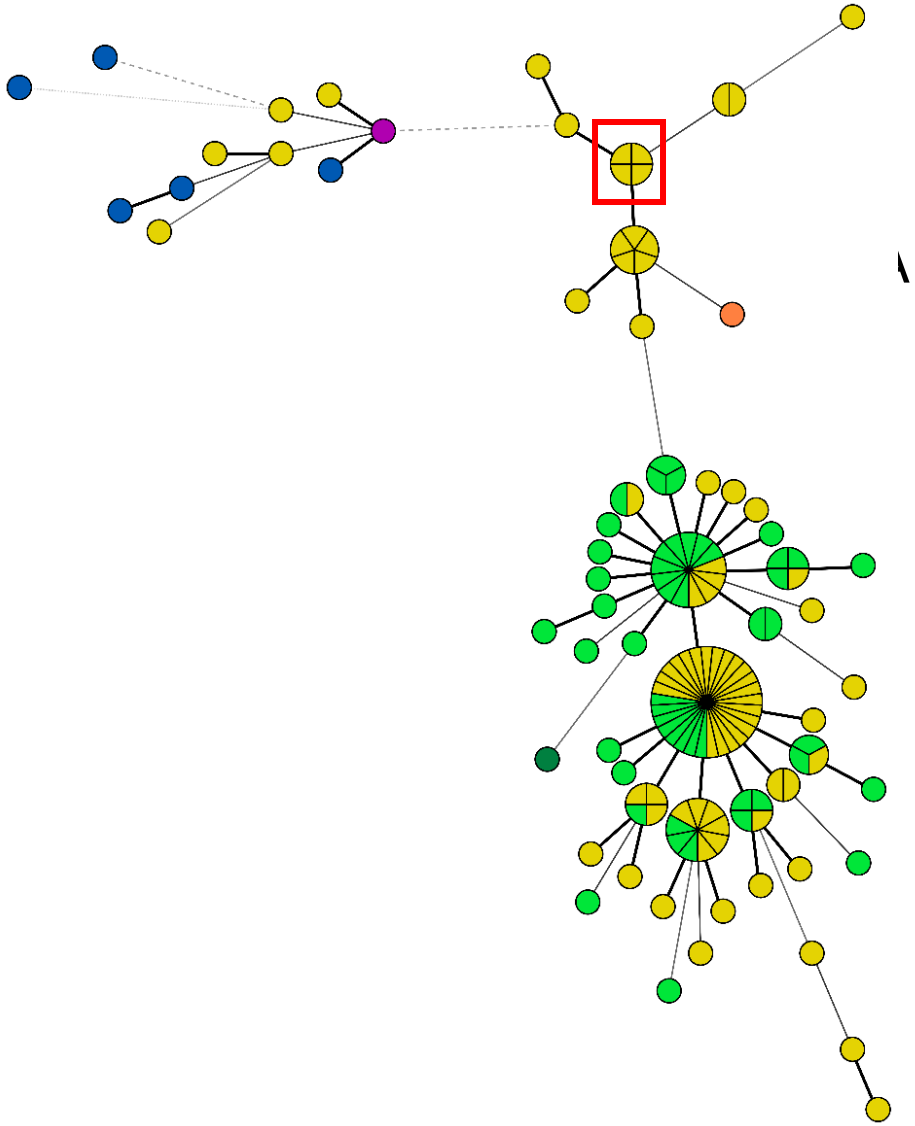


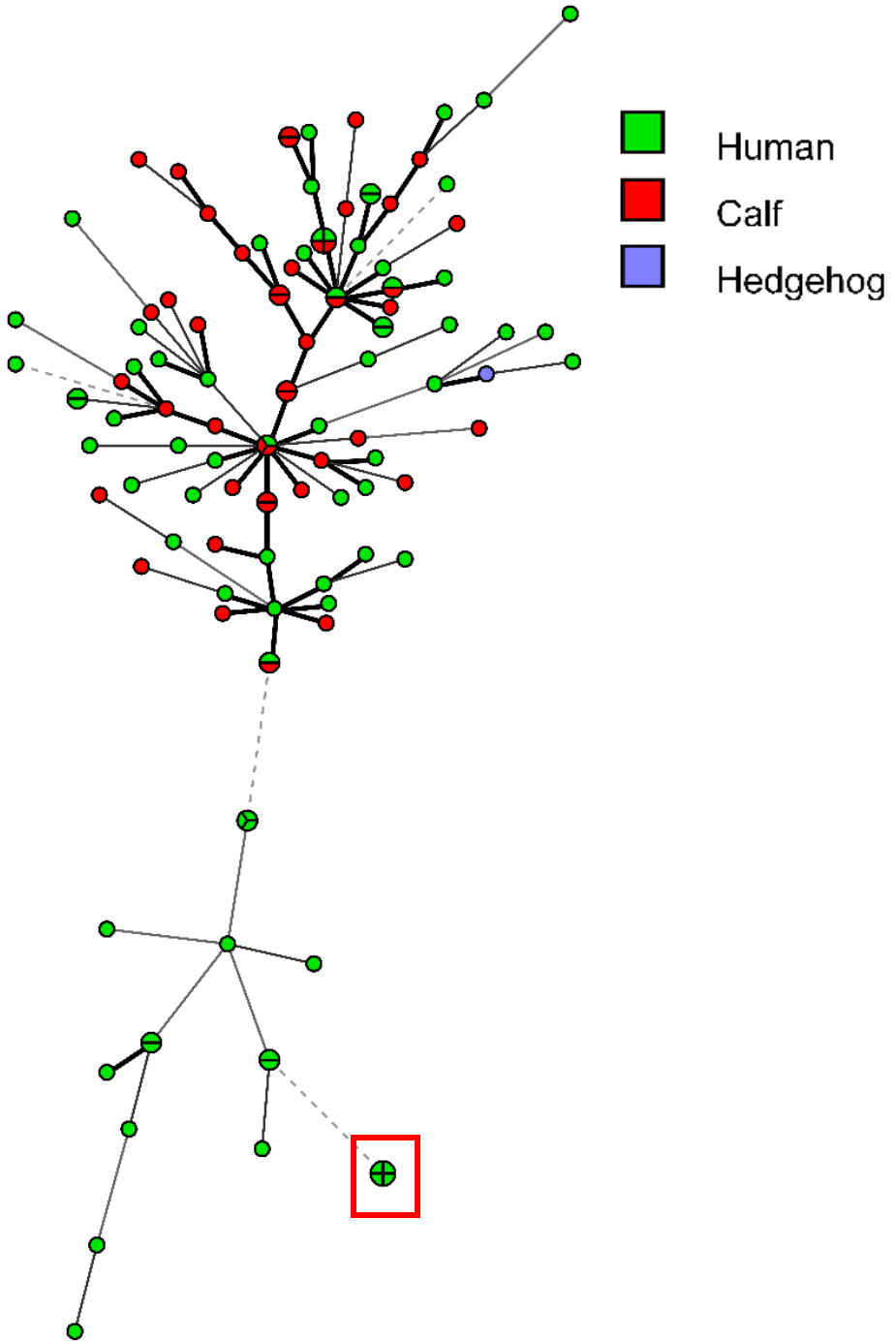
elise.groenen@rivm.nl

Clus

Count
profile

Only c





elise.groenen@rivm.nl

Conclusions

- Both MLST and MLVA detect a human cluster from a potential outbreak
- With MLST we were able to analyze more samples as compared to MLVA (hedgehog cluster) and can be used for more purposes (phylogenetic analyses)
- MLST is the preferred initial method to use in outbreak detection, with MLVA as potential additional method

Acknowledgements

Elise Groenen
Dewi van den Oudenalder
Nimrod de Wit
Pauline Lempens
Sietze Brandes
Diede Wübbels
Chesley van Buuren
Marieke Opsteegh
Lapo Mughini-Gras
Thomas Roodsant

QUESTIONS?

Invitation to contribute samples for analysis

Contact:
kees.van.der.ark@rivm.nl

