Genotyping the zoonotic pathogen *Cryptosporidium parvum* by a new Multi-Locus Sequencing Typing scheme

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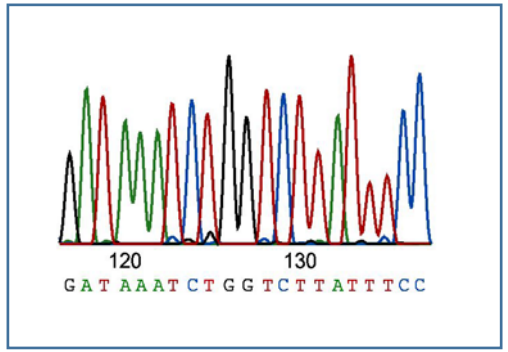
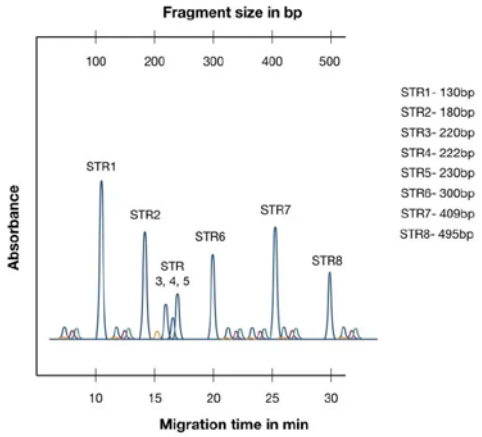
Typing of *Cryptosporidium*: why it is important

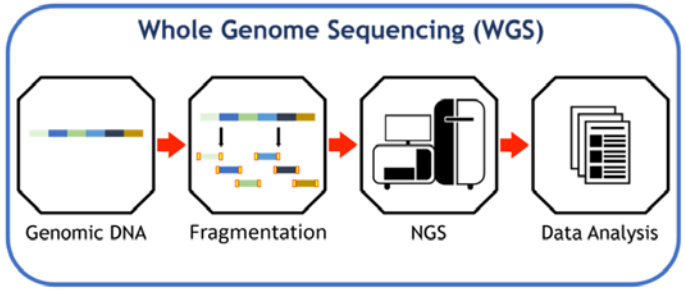
- Epidemiological investigations, including outbreaks
- Source tracking (linking cases to a suspected vehicle of infection)
- Zoonotic transmission
- Parasite population structure
- Linking genotype to phenotype (virulence, environmental stability)

Typing of *Cryptosporidium*: how it is done

- PCR and sequencing of one or more marker genes (*gp60* widely used)
- PCR and fragment typing of VNTR loci
- Whole genome sequencing (still rarely used)

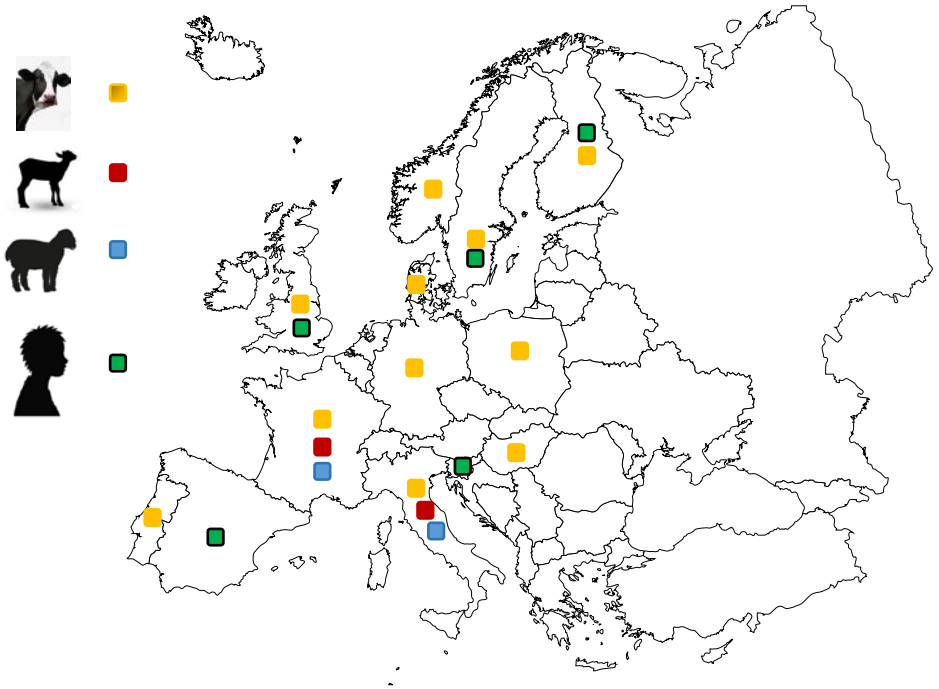






Identification of novel markers: PARADISE project

- We generated135 whole genome sequences of *C. parvum* samples collected across Europe and from the four main hosts (human, cattle, sheep, goat).



Research

Comparative genomics of *Cryptosporidium parvum* reveals the emergence of an outbreak-associated population in Europe and its spread to the United States

Greta Bellinzona,[1] Tiago Nardi,[1] Michele Castelli,[1] Gherard Batisti Biffignandi,[1] Karim Adjou,[2] Martha Betson,[3] Yannick Blanchard,[4] Ioana Bujila,[5] Rachel Chalmers,[6,7] Rebecca Davidson,[8] Nicoletta D'Avino,[9] Tuulia Enbom,[10] Jacinto Gomes,[11] Gregory Karadjian,[2] Christian Klotz,[12] Emma Östlund,[13] Judith Plutzer,[14] Ruska Rimhanen-Finne,[15] Guy Robinson,[6,7] Anna Rosa Sannella,[16] Jacek Sroka,[17] Christen Rune Stensvold,[18] Karin Troell,[13] Paolo Vatta,[16] Barbora Zalewska,[19] Claudio Bandi,[20] Davide Sassera,[1,21] and Simone M. Cacciò[16]

Deriving markers from WGS: in silico workflow

Raw reads from 135 samples.

Read trimming, quality and contamination check.

Extraction of *C. parvum* reads from contaminated samples.

Downsampling to 100 x coverage.

Pre-processed reads

COMPARE pipeline analysis.

Gene annotations and SNPs

Selection of 500 bp gene fragments based on SNP content and Simpson's Index.

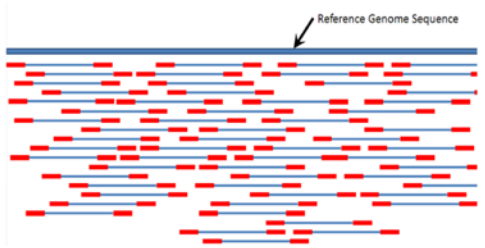
150 marker candidates

Multiple alignment of selected fragments and flanking regions, and primer design.

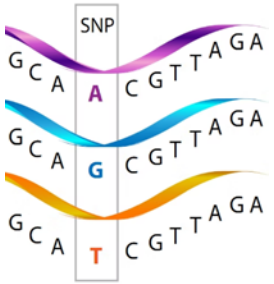
69 marker candidates

In silico evaluation of potential MLST schemes and manual curation.

18 markers selected for PCR testing.



Read mapping

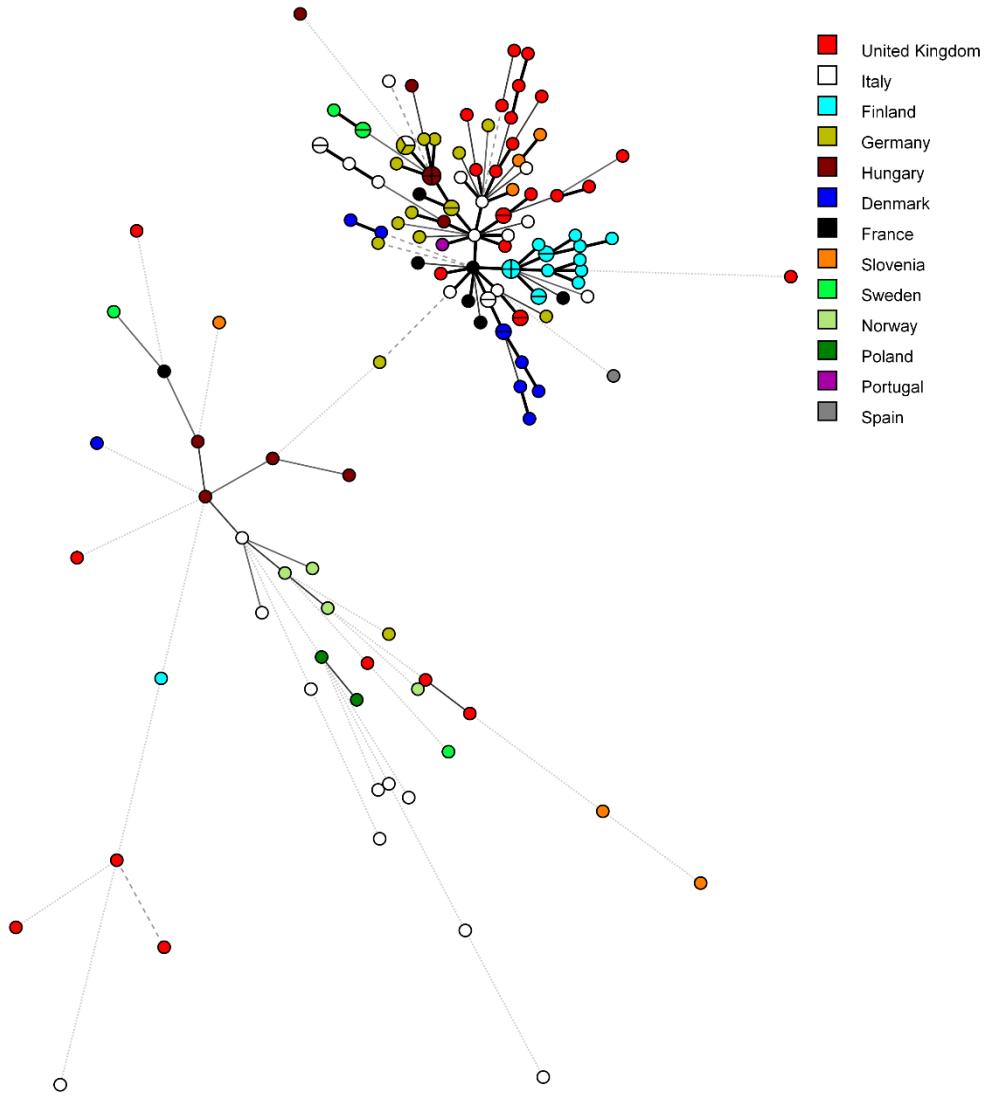


SNP calling

Mulitple alignments

In silico evaluation

Typing with the in silico selected markers



High discrimination of the 135 *C. parvum WGS* samples is obtained when 18 markers are used.

Inclusion of this number of markers in a scheme will require an amplicon sequencing approach

However, we intended to develop a classical PCR and sequecing approach, and we wanted to include 8 markers, one marker per chromosome

Experimental marker selection

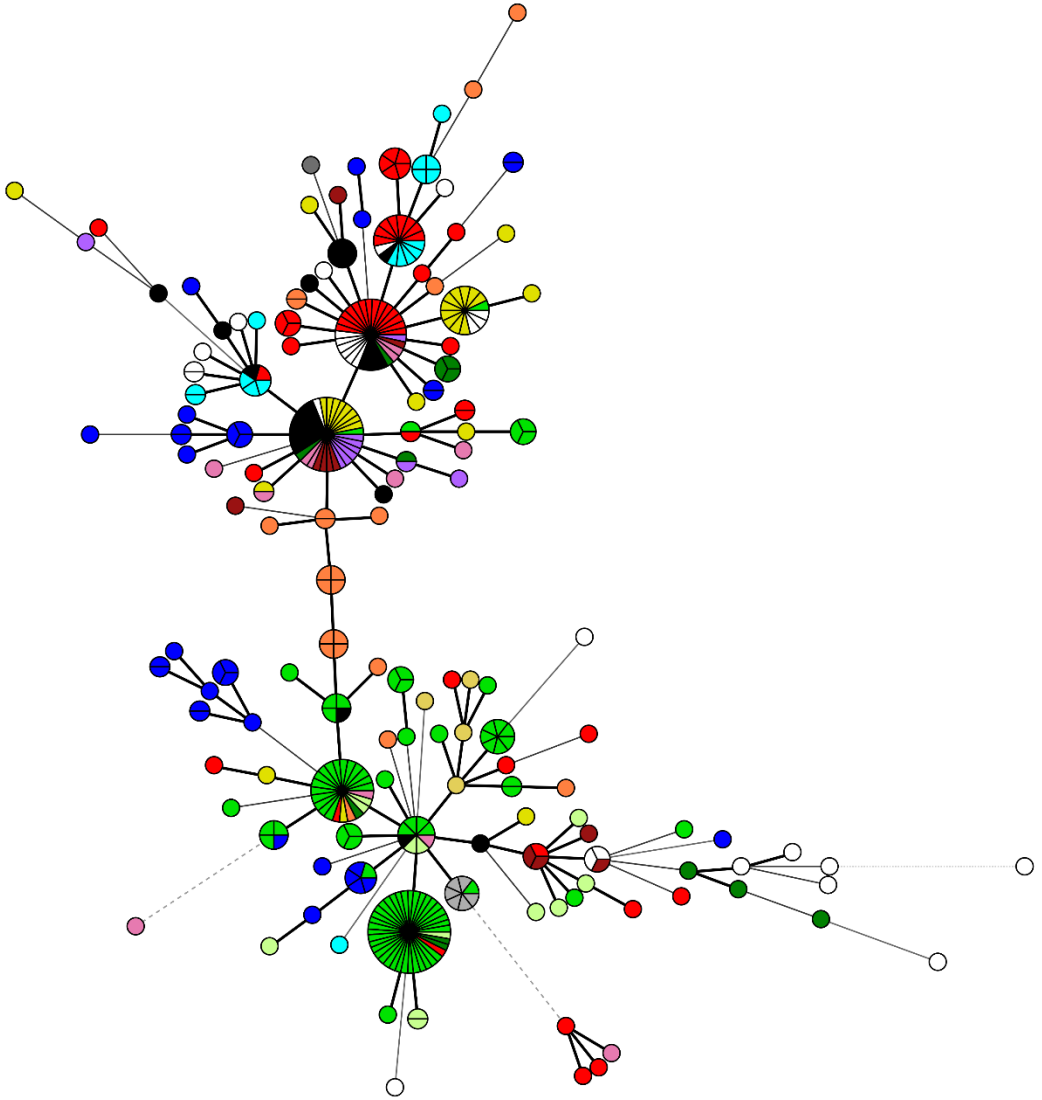
Primer design
PCR set-up
Sanger sequencing

8 markers selected

Final MLST scheme

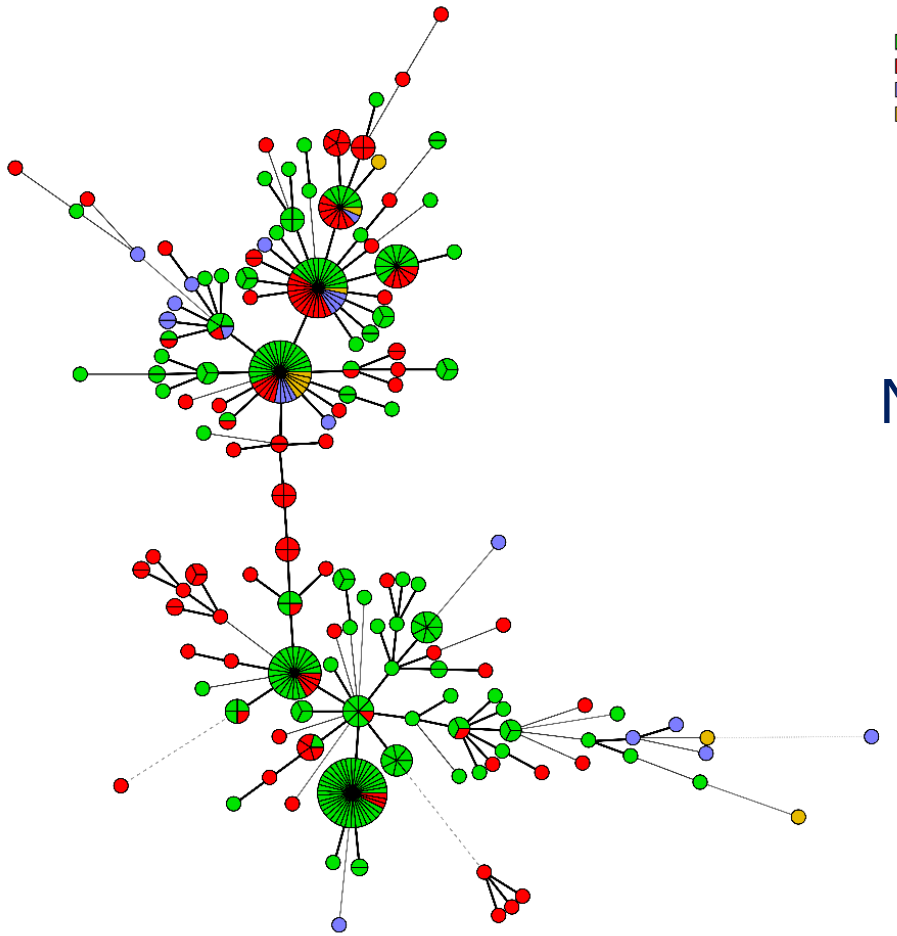
305 samples typed
gp60 gene analysed
Cluster analyses

MLST profiles by country



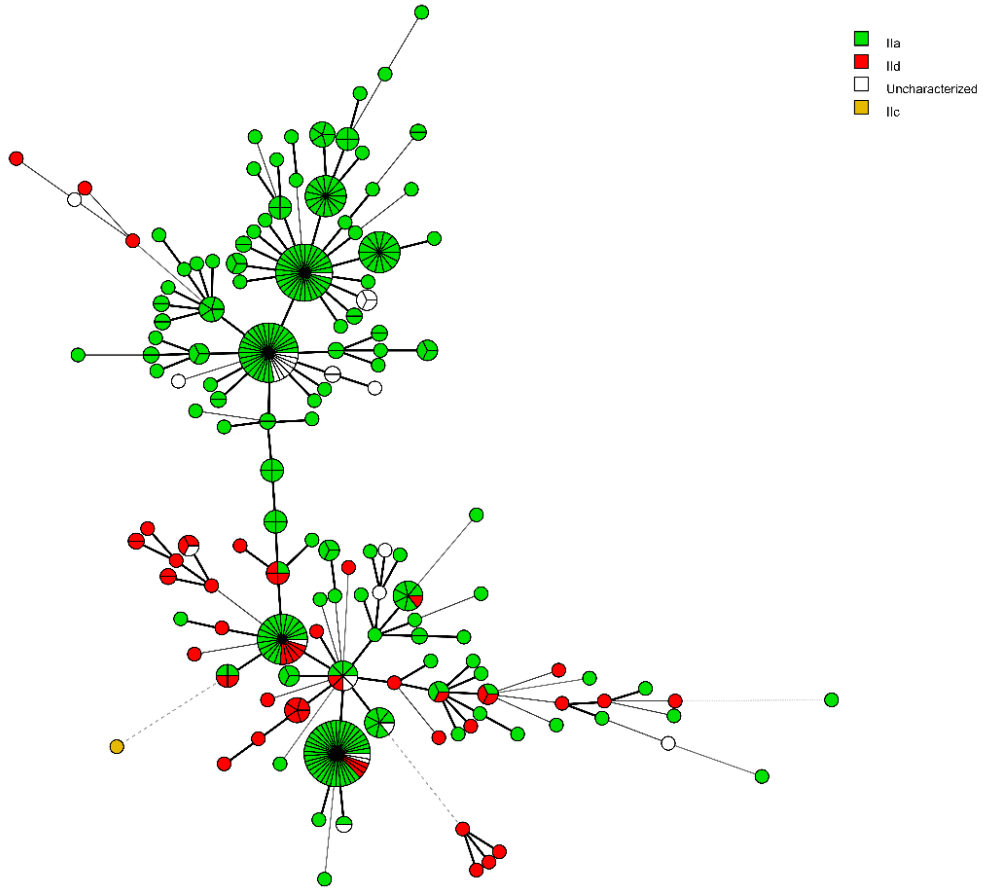
Some MLST profiles are common and were found in different EU countries and in multiple hosts

MLST profiles by host

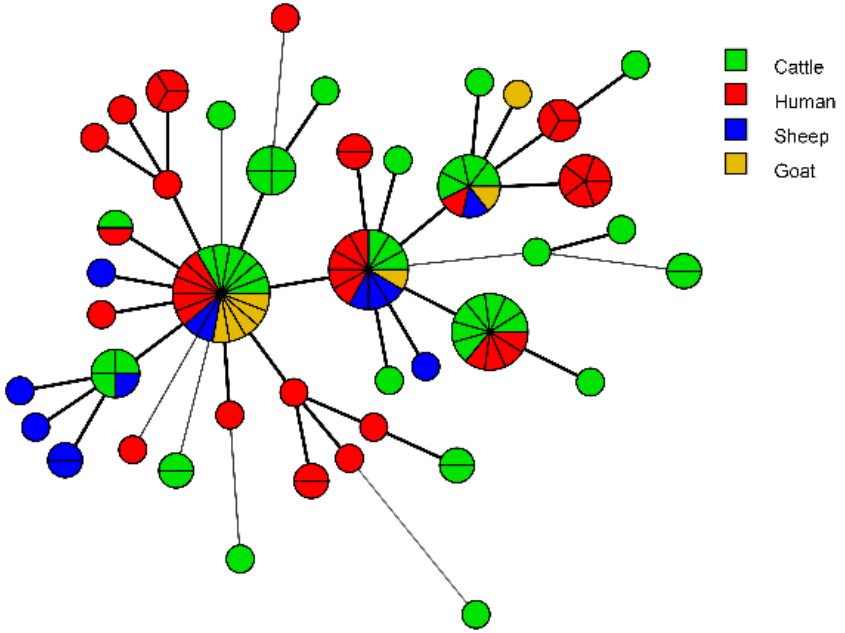


No complete clustering by host

MLST profiles by *gp60* families (IIa and IId)

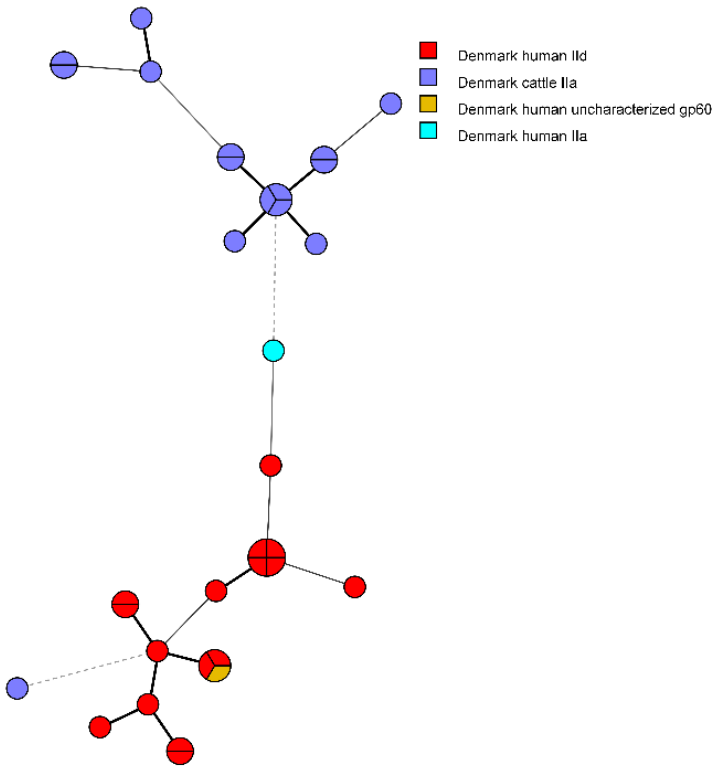


MLST profiles of samples with the IIaA15G2R1 gp60 subtype



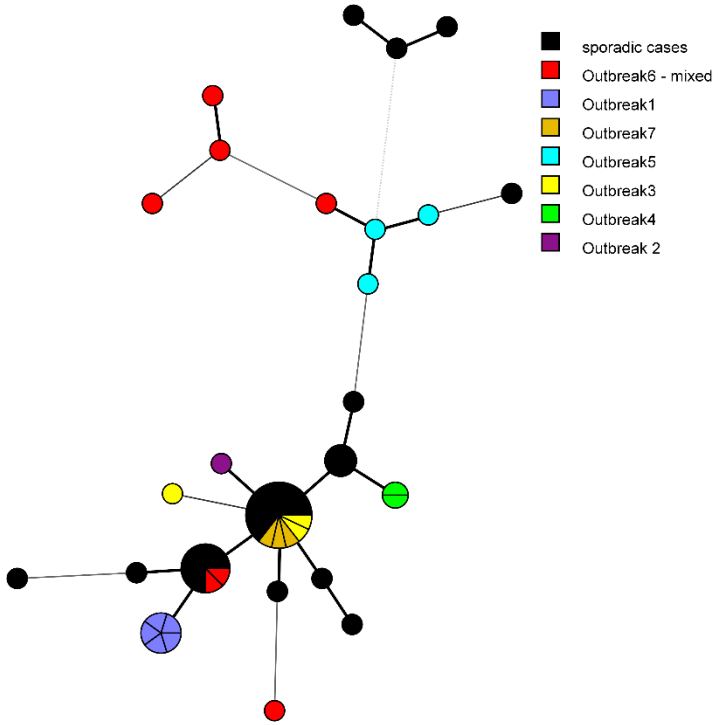
This is the most common profile in Europe, but the MLST scheme clearly shows that it does not correspond to a genetically homogeneous population of parasites (likely due to recombination)

MLST profiles of Danish samples from humans and cattle



Different MLST prfiles associated with human or cattle infection.
No zoonotic transmission? Clearly more data are needed

MLST profiles of UK samples from known outbreaks



Distinct MLST profiles observed only for some outbreaks (number 1 and 4) but not for others (for example, number 5 and 7 have the same profile)

Concluding remarks

- A new MLST scheme has been developed, based on a selection of 8 polymorphic gene markers (one per chromosome) identified by mining 135 whole genome sequences
- The scheme has been tested on a large collection (>300) of *C. parvum* samples from 16 European countries
- No complete clustering of samples by host or geographic origin was observed
- A subset of samples is being analysed with a scheme based on 7 VNTR loci to idnetify the best combination of markers, which could vary depending on the local population structure of the parasite

Acknowledgements: a large collaborative effort!

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And finally published…..

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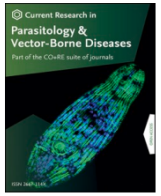
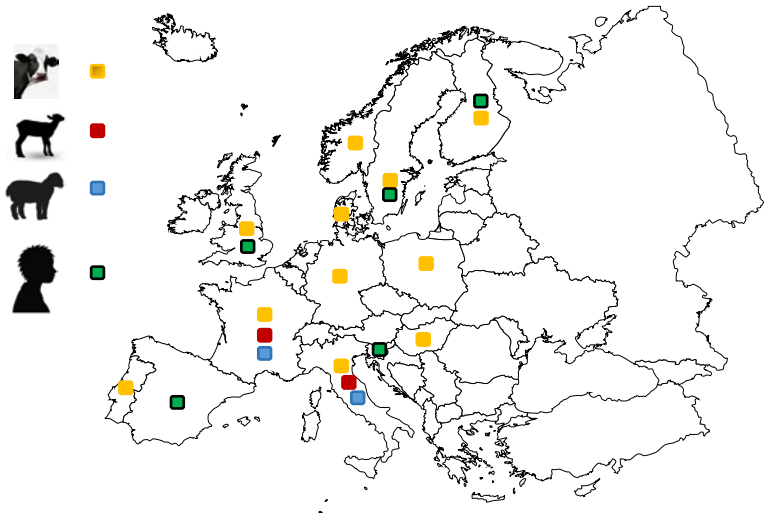


Design, development, and testing of a new multi-locus sequence typing scheme for the zoonotic pathogen *Cryptosporidium parvum*

Karin Troell [a,b,**], Christen Rune Stensvold [c], Anna Rosa Sannella [d], Martha Betson [e], Emma Östlund [a], Rachel M. Chalmers [f,g], Umer Chaudhry [h], Rebecca Davidson [i], Lauren Davies [a], Ralf Ignatius [j], Anton de Jong [a], Gregory Karadjian [k], Karim Adjou [k], Christian Klotz [l], Sokratis Ptochos [b], Guy Robinson [f,g], Jeroen Roelfsema [m], Barbara Soba [n], Jacek Sroka [o], Paolo Vatta [d], Jonas Johansson Wensman [a,p], Simone M. Cacciò [d,*]

Thank you for listening!
Questions?

135 WGS of *C. parvum*



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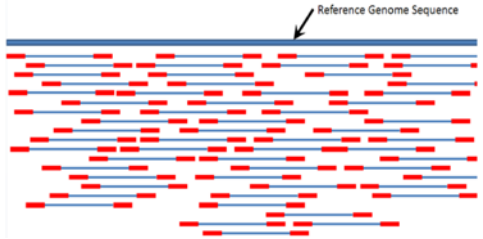

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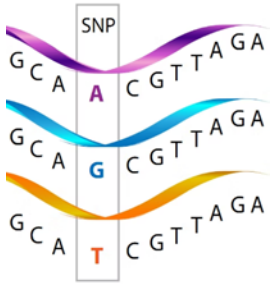

Multiple sequence alignment

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