A genotyping tool to study molecular epidemiology of *Cryptosporidium parvum*

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Cryptosporidium parvum is a protozoan parasite that causes gastro-intestinal disease in both humans and animals. It is highly prevalent in neonatal calves and livestock are often implicated as the source of human infections. Transmission of *C. parvum* between farms can potentially occur:

1. over long distances, spread by movements of infected livestock, or
2. locally, spread by fomites/wildlife/watercourses (indirect transmission) or by contact between animals at contiguous boundaries (direct transmission).

Development and evaluation of genotyping tool

Why genotype *C. parvum*?
- to study transmission dynamics at regional/local levels
- to aid source attribution in outbreak investigations

Multi locus fragment typing (MLFT)
- 7 regions of genome ("markers")
- Markers include regions with variable numbers of DNA "repeats" eg TCA TCA TCA TCA…
- Assign alleles based on size of fragment/no. of repeats
- Assign **multilocus genotype (MLG)** based on allelic profile
- Considered discriminatory in conserved organisms
- Considered to have issues with reproducibility

We evaluated an MFLT tool using epidemiologically related and unrelated *C. parvum* samples from 135 UK calves.
- **Typeability (sensitivity)** ✓
  - No. of samples genotyped/no. of samples attempted = 0.87
- **Specificity** ✓
  - Primers did not amplify other *Cryptosporidium* spp.
- **Repeatability** ✓
  - Results within lab (between runs) were consistent
- **Reproducibility** ✓
  - Between labs, sizes differed but allele calling was consistent
- **Discriminatory ability** ✓
  - Some markers more informative (polymorphic) than others
  - SID 0.92 (good discrimination)

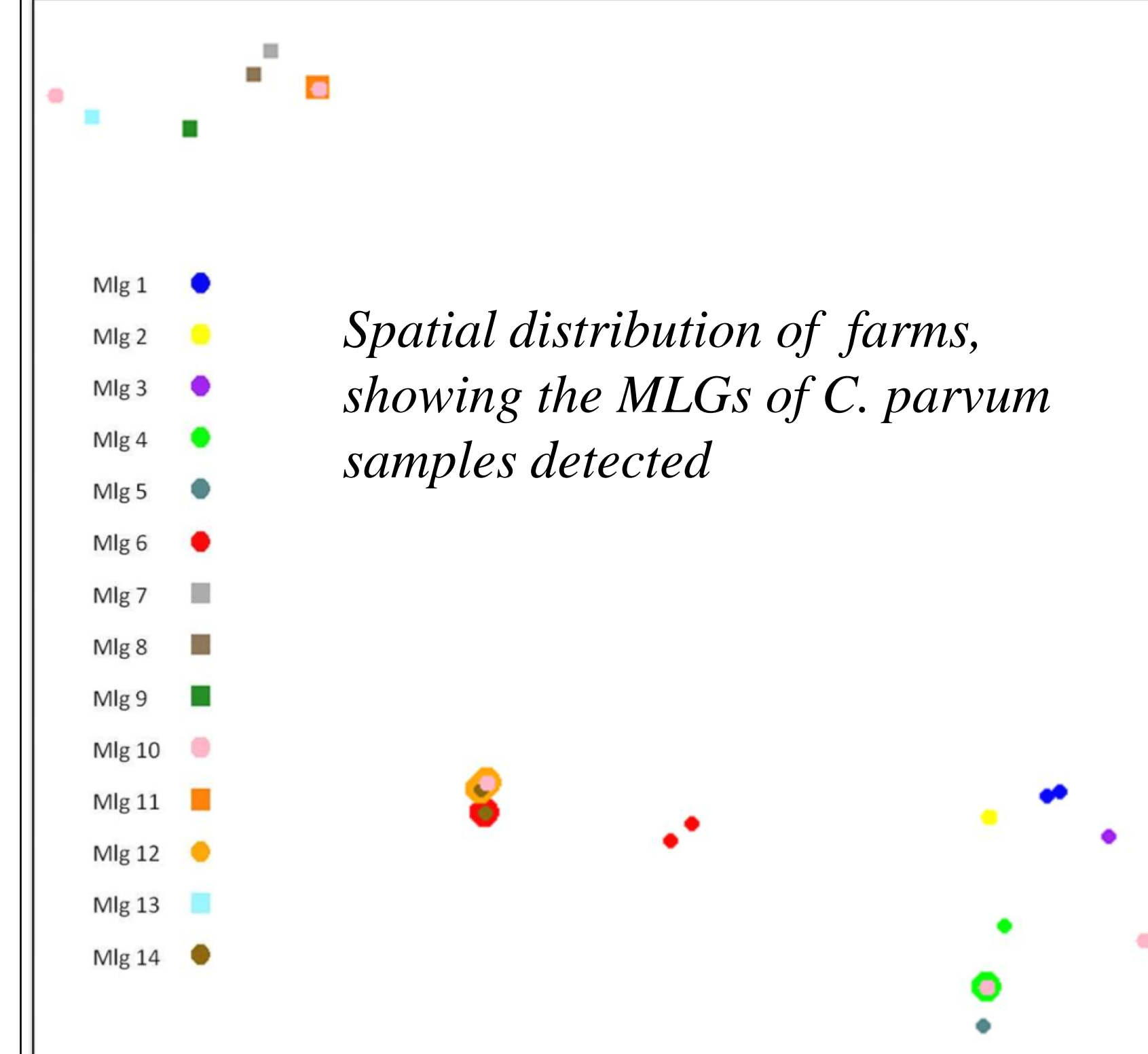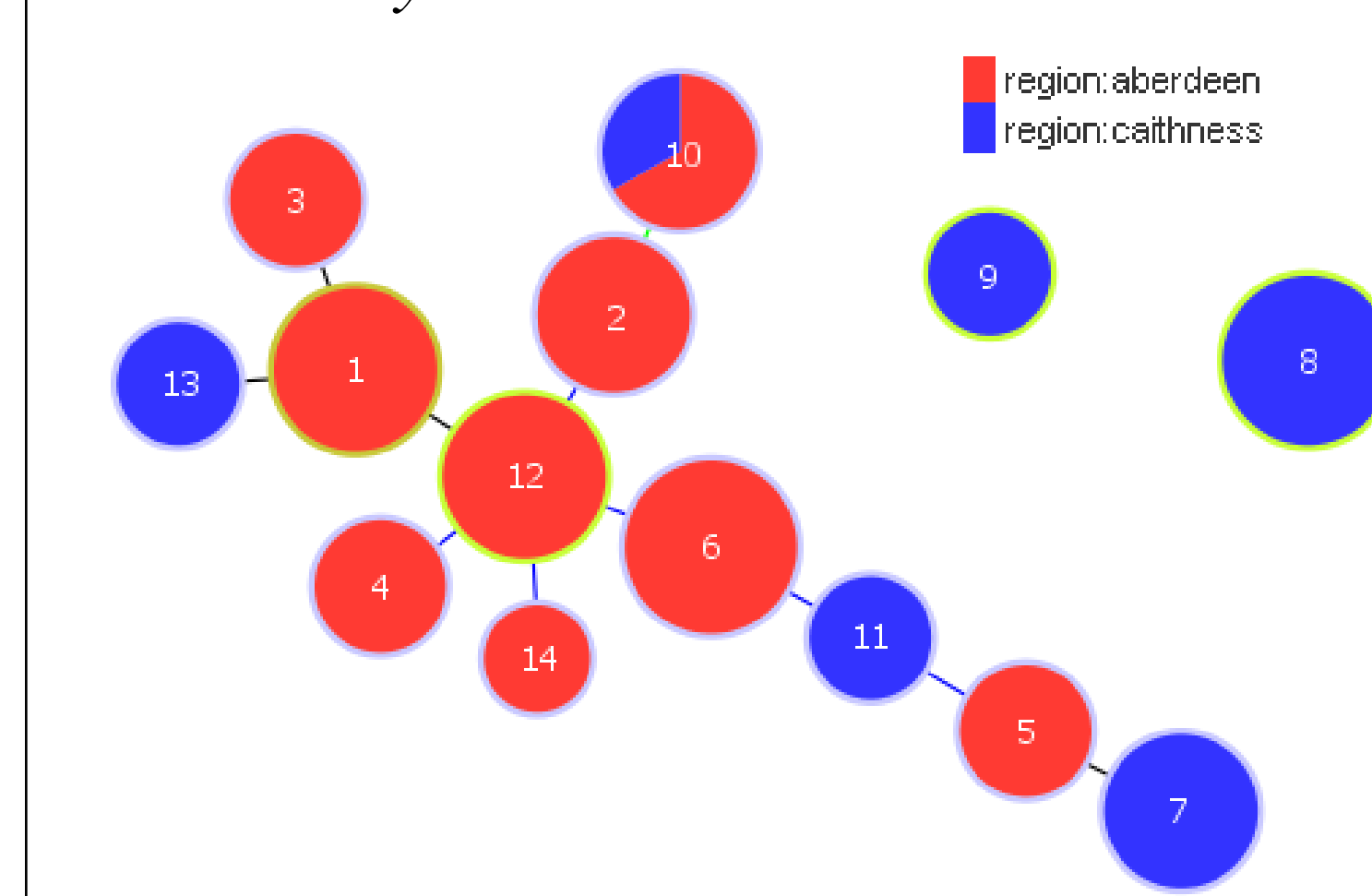## Application of genotyping tool – transmission dynamics between farms

Cryptobeef: a cross-sectional survey of beef farms in Spring 2011, Aberdeenshire and Caithness, Scotland

- *C. parvum* prevalent in young calves; subjected to MFLT
- 14 MLGs in 78 samples from 19 farms
- Most calves (74/78) and farms (14/19) had a single MLG
- 8/14 MLGs were only detected on one farm ("unique")
- Some "common" MLGs found on multiple farms (e.g. MLG 10)
- No association between having a "unique" or "common" MLG and farmer-reported biosecurity practices

- Apparent association between genetic and spatial distance between isolates (Euclidean distance between farm addresses)
- Evidence for local transmission, but:
  - animals may be located far from the holding address
  - farms are often fragmented into parcels of grazing land
  - natural barriers to local transmission may exist
  - farms may trade animals locally

- To further investigate this, we are collating movement data and location of land parcels used for grazing cattle.

Minimum spanning tree depicting genetic relationship between Cryptobeef C. parvum isolates. Each dot represents a unique MLG; diameter of the dot is proportional to the number of isolates. Single-locus variants are connected by lines.

region:aberdeen
region:caithness

Spatial distribution of farms, showing the MLGs of C. parvum samples detected

Mlg 1, Mlg 2, Mlg 3, Mlg 4, Mlg 5, Mlg 6, Mlg 7, Mlg 8, Mlg 9, Mlg 10, Mlg 11, Mlg 12, Mlg 13, Mlg 14

Outcome	Explanatory variable	Aberdeenshire (n=117)				Aberdeenshire and Caithness (n=249)			
		Cases	Controls	OR (95% CI)	P value	Cases	Controls	OR (95% CI)	P value
Farms share identical MLGs	Spatial dist (Km)	9	108	0.95 (0.90-0.99)	0.017	13	236	0.99 (0.98-1.00)	0.048
Farms have MLGs that share at least 6/7 alleles	Spatial dist (Km)	45	72	0.98 (0.95-1.00)	0.049	65	184	0.99 (0.99-1.00)	0.002

Results of logistic regression exploring the relationship between genetic and spatial distance between isolates

Conclusions
- MFLT is useful for local epidemiological questions in bovine *C. parvum*
- These markers have also shown to have good typeability and discrimination in human *C. parvum*
- We are currently collaborating with public health bodies to validate an integrated typing scheme

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