A new multilocus subtyping scheme for *Cryptosporidium parvum*

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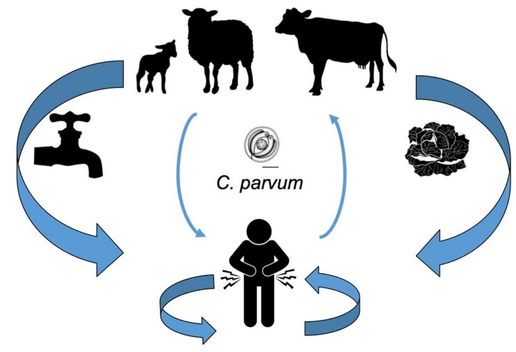


Aim To improve the resolution and application of *Cryptosporidium parvum* subtyping.

Background

Cryptosporidium is an important cause of gastroenteritis, with up to 6000 reported cases annually in the UK. However, cases and outbreaks are under-ascertained.

Species identification is a reference test, and subtyping during outbreaks is based on sequencing the *gp60* gene.

Cryptosporidium parvum has strongly emerged as the predominant species during the COVID-19 pandemic.

To improve outbreak investigations a novel genotyping scheme for *C. parvum*, based on multilocus variable number of tandem repeats analysis (MLVA), was validated.

Method validation

PCRs were developed for seven markers on different chromosomes, or distant if on the same chromosome and tested on a validation panel:

Validation panel 259 samples

- 108 sporadic human cases
- 91 livestock cases
- 5 outbreaks; 60 cases

Amplicons were sized by capillary electrophoresis and calibrated against sequenced reference standards.

A MLVA profile was compiled by listing the calculated number of repeats for each marker in chromosomal order expressed as a numerical string e.g. 6-14-5-7-27-28-16.

A subset of samples was used in an inter-laboratory trial.

Results

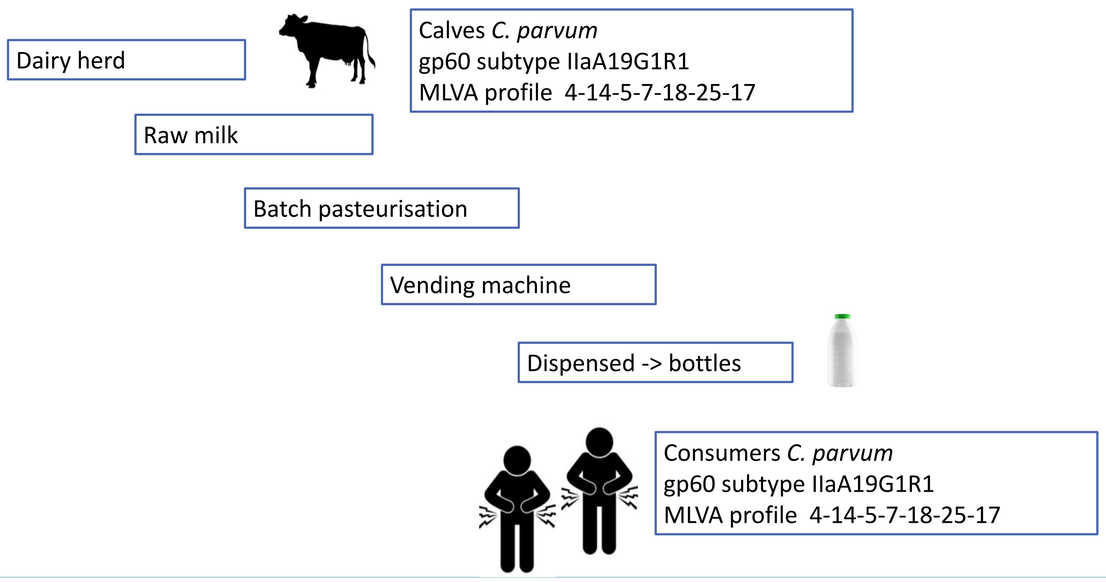
- Typability of 259 samples = 85%
- Discriminatory power for 136 unrelated samples = 0.99, compared with 0.74 for gp60 sequencing.
- Epidemiological concordance was demonstrated in historical outbreaks.
- Reproducibility was demonstrated by sample exchange between three laboratories (CRU, Scottish Microbiology Reference Laboratories, and Moredun Research Institute).

Application

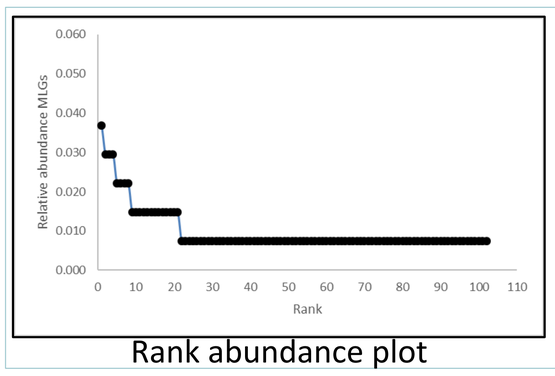
Investigation of an outbreak associated with drinking milk from an on-farm vending machine in south west England, December 2020-January 2021.

C. parvum-positive stools from cases and, in the absence of milk samples and a standard method for testing milk, faeces from a *C. parvum*-positive calf were analysed.

Case and calf samples were indistinguishable at all seven loci.



Hypothesis generation



Rank abundance plot

Among the 136 epidemiologically unrelated samples in the validation panel, the majority of MLGs (81/102, 79%) were unique, indicating that clusters identified from MLVA profiles could help identify outbreaks.

The hypothesis is that truly un-related isolates have greater diversity than epidemiologically-related ones. Investigation is underway.

Conclusion

Stronger evidence can be obtained in outbreak investigations by improved microbiological approaches. MLVA subtyping will be offered by the Cryptosporidium Reference Unit as a core service in outbreaks.

There is also likely much to be gained from application of MLVA to identify outbreaks that might otherwise be missed. An HPRU GI pump priming project is underway to explore whether the genetic diversity of *C. parvum* identified by MLVA can identify epidemiological clusters for further investigation at a local and/or national level.

There is potential for MLVA surveillance to be delivered by the CRU as a core service. UKAS accreditation is applied for.

Acknowledgements

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