Molecular epidemiology of *Cryptosporidium* in cattle – can contact networks explain subtype variation?

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Background:

The protozoan parasite *Cryptosporidium* can cause severe diarrhoea, dehydration and sometimes death in calves, particularly neonates. *C.parvum* has been responsible for clinical disease in both animals and humans and is recognised as a zoonotic pathogen.

Animals acquire infection by ingesting oocysts from the faeces of affected individuals; oocysts are resistant to many disinfectants and survive well in the environment, thus parasite eradication from farms can be difficult.

Molecular techniques have been used previously to study genetic variation below species level in order to identify pathogen subtypes; to date the majority of studies have focused on clinically affected individuals (humans and cattle) over wide geographical areas.

To fully understand the transmission dynamics of this pathogen, it is crucial to examine subspecies diversity in the general calf population in discrete geographical areas. Genetic variability combined with epidemiological contact data may highlight the most likely transmission routes and those contacts that carry the most risk.

Methods:

Calf faecal samples were collected from cattle farms in a 100km^2 area of north-west England. Epidemiological contact data was collected via interview-based questionnaires from these farms, identifying the direct and indirect contact types that exist in this area.

Samples were subject to Polymerase Chain Reaction (PCR) amplification using 5 micro/minisatellite primers targeting highly variable regions of parasite DNA. Amplified DNA fragment lengths along all 5 regions were identified and multilocus genotypes (MLG), or subtypes, assigned to each sample. Clustering of multilocus genotypes to generate a dendrogram of similarities between samples was carried out using Clustering Calculator (www.biology.ualberta.ca/jbrzusto/cluster.php).

Contact data were converted into matrices in Ucinet (www.analytictech.com). Relationships between farm subtypes and contact networks were examined using Quadratic Assignment Procedure (QAP) correlation and multiple regression QAP (MRQAP) via the Double Dekker Semi-Partialling method (based on least squares regression and permutation methods for significance) in Ucinet.

Genotypes:

Organisms were isolated from 55/215 samples originating from 20/41 farms; considerable genetic diversity was seen between holdings when compared to previous studies involving clinically affected animals. Amplification along all 5 regions was possible in 46 samples (20 farms). Using *Cryptosporidium*-specific primers, 45 samples were successfully sequenced as *C.parvum* (1 unknown).

A total of 29 multilocus genotypes were found (Figure 1).

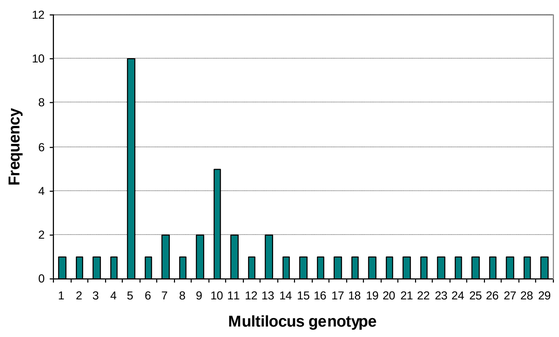


Figure 1: Multilocus genotypes of *Cryptosporidium* parasites from calf faecal samples

Dendrogram:

A dendrogram was generated using Ward's clustering method and Jaccard's similarity coefficient to cluster the MLGs (Figure 2). Broadly, the samples were grouped into two clusters. 10 samples from 6 farms exhibited the same genotype, with another 5 samples from 2 farms also having identical genotypes.

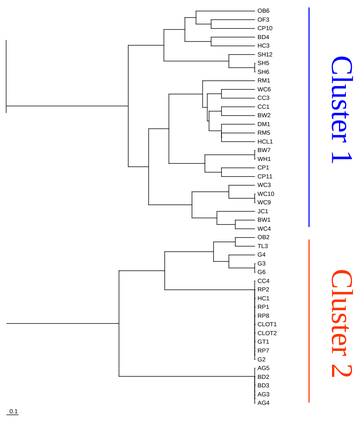


Figure 2: Dendrogram of multilocus genotypes

QAP correlation and univariable regression:

Similarities between samples were reduced to a single value according to the number of common alleles (0 if no common alleles, 1 if all 5 alleles the same). Multiple samples from the same farm were reduced to a single farm value by using the maximum similarity occurring between premises. As epidemiological contact data was available for 17 of the 20 farms sampled, 17 by 17 matrices were constructed for the QAP analysis.

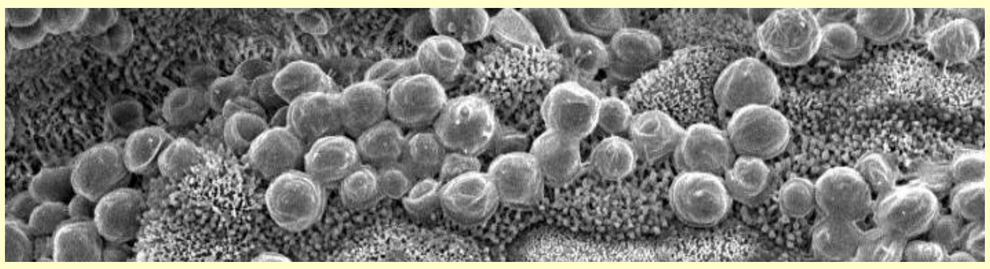
Factors with significance P<0.26 in the univariable QAP regression were selected to be included in the final multivariable regression model (13 factors in total). Significant QAP correlations (P<0.05) were seen between the distance between farms network and the contiguous neighbour, social interaction and watercourse networks; in the final multivariable model only distance between farms was included.

QAP multivariable regression:

Factors that were significantly similar in the final model can be seen below (Table 1).

Table 1: Significant factors (P<0.05) in a multivariable model of *Cryptosporidium* subtype variation versus between-farm contacts

Factor	Standardised coefficient	Significance
Use of dealers	-0.32	0.005
Use of markets	-0.21	0.04
Centred distance^2 (m^2) between farms	0.22	0.02
Centred distance (m) between farms	-0.09	0.1
Dairy enterprise	0.21	0.04
Government veterinarian visits	0.17	0.048



Conclusion:

Farms that trade with markets and dealers are more likely to have dissimilar subtypes; accumulation of novel subtypes may occur through purchase of stock from diverse sources or via fomite transmission on vehicles. Farms that are contiguous neighbours are more likely to share equipment, socialise and trade animals in similar ways (data not shown) potentially increasing the likelihood of pathogen subtype similarities; it is possible that distance between farms is a proxy for such contacts. Dairies are more likely to have similar subtypes perhaps due to similar animal trade and indirect contacts.

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