

# Cryptosporidium spp. in Cattle in a Jamaican Watershed

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## Background

- *Cryptosporidium* is a protozoan parasite associated with gastrointestinal illness in humans and a wide range of vertebrate animals.
- Cattle, particularly calves, are widely recognized as major reservoirs of zoonotic *Cryptosporidium spp.*
- In the Caribbean region, molecular characterization of *Cryptosporidium spp.* in cattle is limited and their zoonotic potential is poorly understood.

## Objectives

- To perform an exploratory investigation on the presence and molecular characterization of *Cryptosporidium spp.* in dairy and beef cattle in a Jamaican watershed and determine whether cattle are potential zoonotic reservoirs for human infection on the island.

## Methods

- A total of 119 fecal specimens were collected from 60 dairy and 59 beef cattle from 10 farms in the watershed (Fig 1).
- Approximate age and absence/presence of diarrhea was recorded from each specimen.
- Phosphate-buffered saline (PBS)-ether sedimentation was performed to concentrate potential *Cryptosporidium* oocysts from individual specimens.
- Initial screening for *Cryptosporidium* was performed by modified acid-fast (MAF) staining microscopy, followed by conventional and nested PCR amplification of a polymorphic locus on the *18S rRNA* gene and sequencing.
- A phylogenetic tree was constructed using neighbour-joining in MEGA 11, based on Kimura's two-parameter model with bootstrap values obtained from 1000 replicates.
- Further subtyping analysis of PCR-positive isolates was performed by *gp60* nested PCR and sequencing.
- The *gp60* subtype was identified based on family designation and the number of trinucleotide repeats (TCA or TCG) in the sequence .

## Results

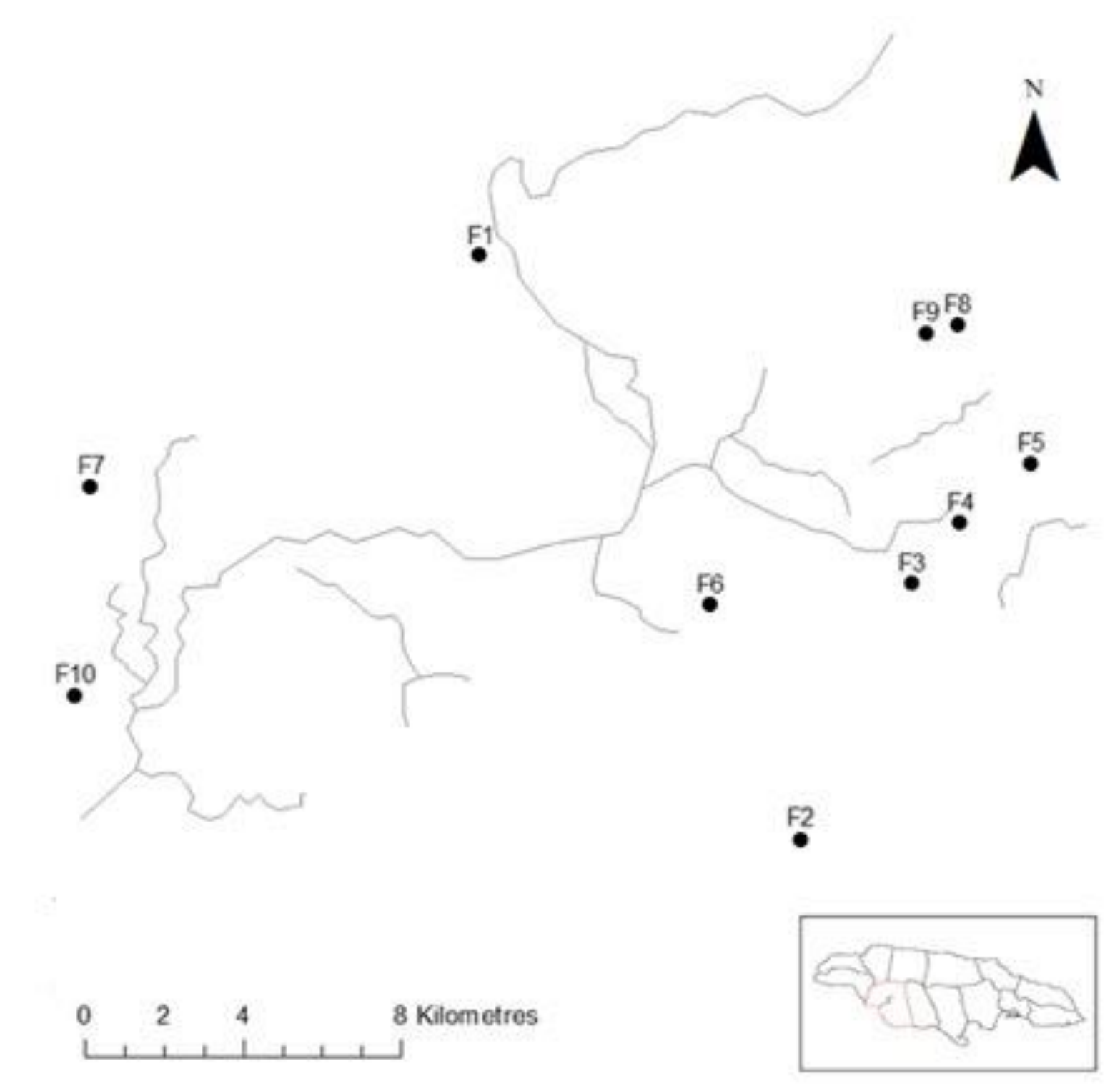


Fig 1. Map illustrating sampling sites in agricultural watershed in St. Elizabeth, Jamaica

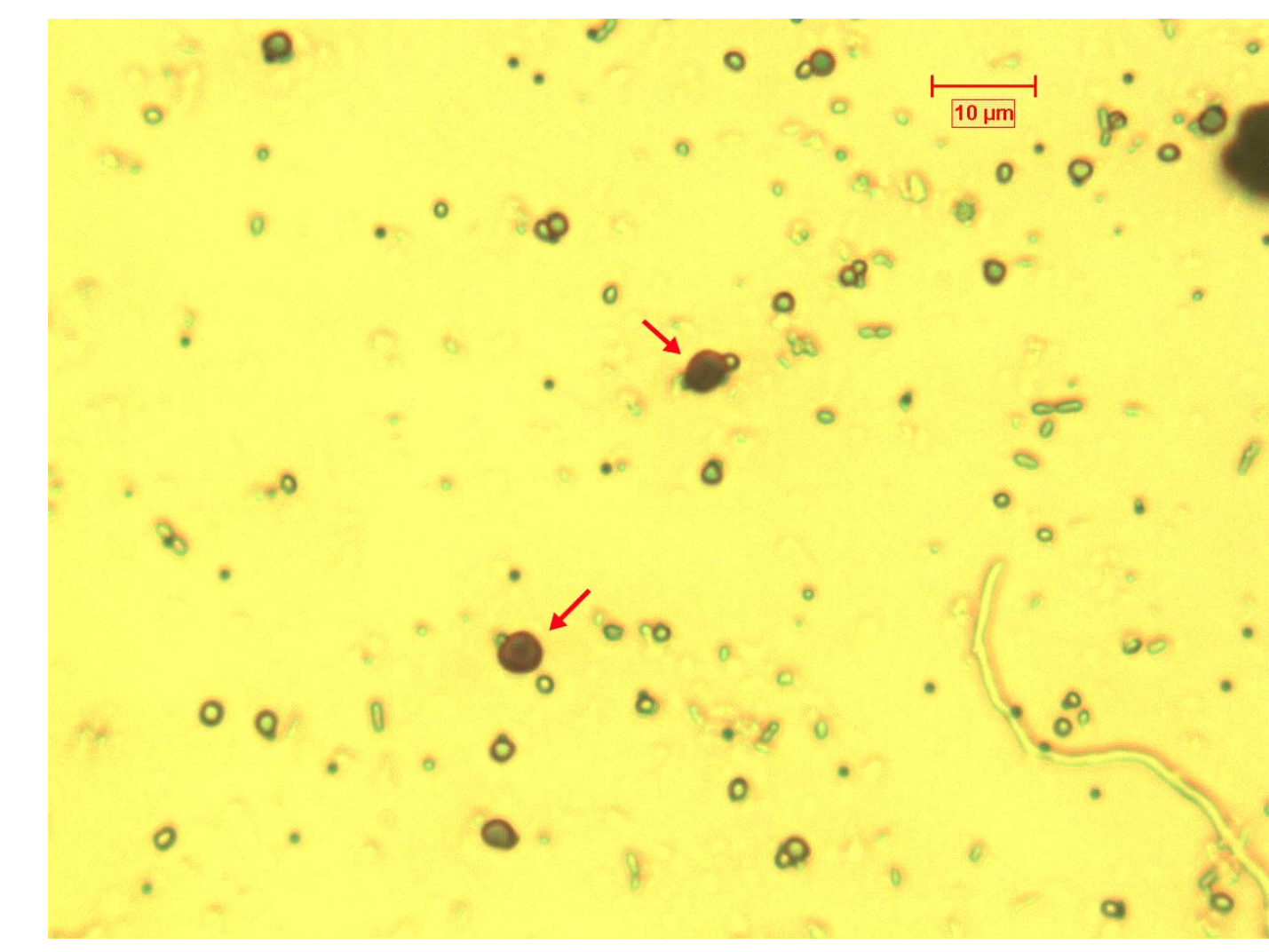


Fig 2. Representative images of *Cryptosporidium* oocysts detected from cattle sample 56DaCw by PBS-ether sedimentation and MAF staining. The Ward's® Chemistry Acid-Fast Stain Kit was used to stain oocysts, as per the CDC modified acid-fast staining procedure. Oocysts have a well-formed wall with diameter of approximately 4-5 µm. 100X with immersion oil.

Table 1 *Cryptosporidium spp.* identified from cattle by PCR and sequence analysis of the *18S rRNA* and *gp60* loci

Farm ID	Sample ID	GenBank accession no.	18S rRNA		Gp60	
			Species (% identity)	GenBank accession no.	Species (subtype ID)	Reference
F3	105Da <sup>b,c</sup>		<i>C. hominis</i> (82 %)	MN836824; MK982514	<i>C. hominis</i> (IbA9G2)	Razakandrainibe et al. 2018
F4	8Be <sup>a,c</sup> 13Da 40Da <sup>c</sup> 46Be	OK361786	<i>C. parvum</i> (90 %)	MW947436		
F7	53Da <sup>a,c</sup>	OK325584	<i>C. parvum</i> (99 %)	MF074701; AB513881		
F9	56Da <sup>b,c</sup> 34Da <sup>b,c</sup>	OK310618	<i>C. parvum</i> (99 %)	MF074701; AB513881		
F10	78Da <sup>b</sup> 64Be <sup>c</sup>					

<sup>a</sup> pre-weaned calf ≤ 6 months  
<sup>b</sup> weaned calf ≤ 2 years > 6 months  
<sup>c</sup> diarrhetic symptoms

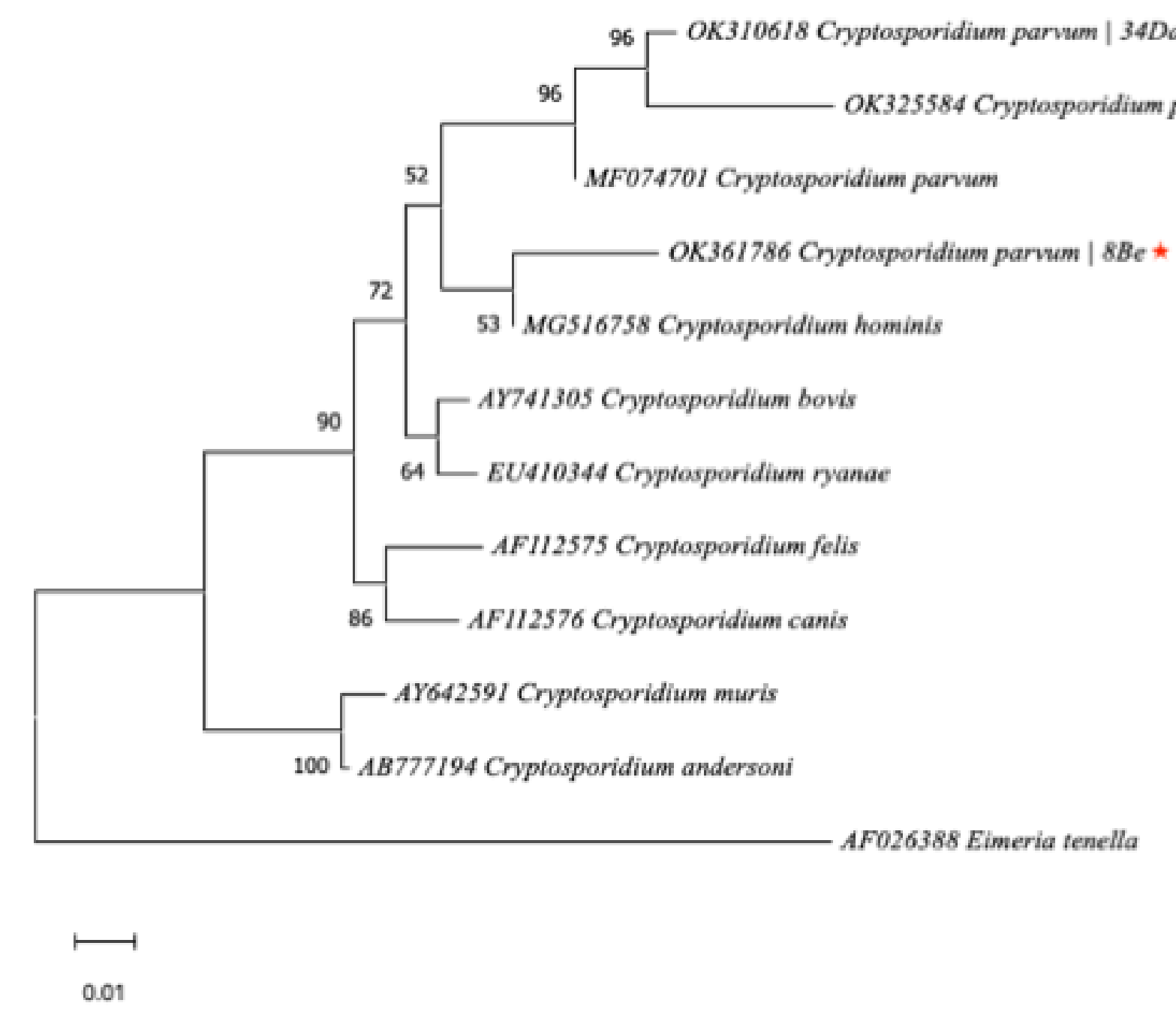


Fig 3. Phylogenetic tree of *Cryptosporidium spp.* based on partial *18S rRNA* nucleotide sequences. The tree was rooted with the *Eimeria tenella* outgroup specie. Each species is marked with an identifying accession number. Sequences generated in the present study (≥90% rRNA homology consensus) are indicated with red stars. Bar = 0.01 substitutions per site.

## Results

- Of the 119 cattle fecal specimens, 10 (8 %) were confirmed positive for *Cryptosporidium* infection by PCR.
- Of the 10 PCR-positive samples, 7 were from cattle presenting symptoms of diarrhea at the time of sample collection; 5 were from calves ≤ 2 years of age, including two pre-weaned calves ≤ 6 months (Table 1); and 7 were from dairy cattle.
- Sequence analysis of the *18S rRNA* gene locus, identified *C. parvum* from one beef calf (no. Be8) and two dairy calves (nos. 34Da and 53Da). Phylogenetic analysis confirmed that *C. parvum* isolates, 34Da and 53Da, share a 96 % nucleotide sequence identity (Fig 3).
- Further sequence analysis of the *gp60* gene, identified the *C. hominis* IbA9G2 subtype in dairy calf no.105Da, previously reported in an impaired calf (Razakandrainibe et al., 2018).

## Conclusions

- This study provides first insight into *Cryptosporidium spp.* infecting Jamaican dairy and beef cattle. Preliminary data suggest cattle, particularly impaired dairy calves, may be important reservoirs of zoonotic *Cryptosporidium spp.* However, the low prevalence of characterized isolates cannot conclude any correlation.
- Additional studies, with a larger number of PCR-positive isolates from calves are necessary to provide critical information about geographically distinct, clonal genotypes of *Cryptosporidium spp.*, which have potential to cause zoonosis in the country.
- With meaningful interpretations of *Cryptosporidium* population structures, useful databases can be built through analysis of well-planned sets of human and environmental samples.

## Acknowledgements

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