

Supplemental Material: Risk Factors Associated with Common *Cryptosporidium* Species and Genotypes in Tennessee, 2018–2023

Supplemental Laboratory Methods:

Positive *Cryptosporidium* stool specimens are submitted to the Tennessee Department of Health Division of Laboratory Services for confirmatory testing and subsequent speciation. *Cryptosporidium* testing was initially validated to assess performance characteristics of the assay, including precision. Testing is intended for public health surveillance purposes only.

Specimen submissions for testing must meet specimen acceptance criteria to undergo testing. These criteria include 1) minimum specimen volume, temperature storage/transport conditions, and acceptable specimen transport media (i.e., Cary-Blair, C&S, Zinc PVA, etc.) found on the laboratory's test directory of services at <https://www.tn.gov/content/tn/health/health-program-areas/lab/directory-of-services/crypto.html>. All specimen types must adhere to the collection, temperature and submission limitation requirements for testing to transpire at the state laboratory (table).

Preservation		Storage/Transport	Submission time limitation
Unfixed	Frozen	-20 - -70°C	None
	Fresh	2-8°C	<1 month from collection
Fixed	Cary-Blair media	2-30°C	<30 days from collection
	C&S culture transport media	2-30°C	<30 days from collection
	Zinc PVA	17-25°C	<30 days from collection
	Total Fix media	17-25°C	<30 days from collection
	Potassium dichromate	2-30°C	<30 days from collection
	Amies Transport media	2-30°C	<30 days from collection
	Stuart Transport media	2-30°C	<30 days from collection

These criteria were established during test method validation and ensure accurate and reproducible results. Additionally, pre-analytical measurements are considered including use of a state-wide courier for environmental controlled transport of specimen as well as verifying temperatures and environmental acceptance criteria using temperature monitoring at the time of specimen receipt.

Initial testing distinguishes between *Cryptosporidium hominis/parvum* or *Cryptosporidium* species (not *C. hominis/parvum*). DNA is then extracted from fecal specimens and analyzed by the gp60 nested PCR method to differentiate *Cryptosporidium* at the subtype level. Primers specific for *C. parvum* or *C. hominis* generate the PCR product of approximately 800 bp, with some length differences for different subtypes. DNA extracts from *Cryptosporidium* PCR confirmed specimens are sent to CDC for Sanger sequencing.

Supplemental Table 1: Questions asked by both the FoodNet 2018–2019 Population Survey and the Tennessee cryptosporidiosis case report form (CRF).

Variable Name	FoodNet Population Survey	Cryptosporidiosis CRF
Diarrhea Contact	In the past 7 days, did you/your child have a household member with diarrhea, or did you/your child spend any time with someone with diarrhea?	Contact with any other persons having diarrhea [in the 7 days prior to illness]?
Cat	In the past 7 days, did you/your child have any contact with a cat?	Did the patient come into contact with any animals [in the 7 days prior to illness]?
Dog	In the past 7 days, did you/your child have any contact with a dog?	Did the patient come into contact with any animals [in the 7 days prior to illness]?
Goat/Cow/Sheep	In the past 7 days, did you/your child have any contact with a cow, sheep, or goat?	Did the patient come into contact with any animals [in the 7 days prior to illness]?
Chicken/Turkey	In the past 7 days, did you/your child have any contact with live poultry of any age, such as chicken or turkey?	Did the patient come into contact with any animals [in the 7 days prior to illness]?
Other Bird	In the past 7 days, did you/your child have any contact with other birds, such as pet birds or wild birds, such as parrots or pigeons?	Did the patient come into contact with any animals [in the 7 days prior to illness]?
Farm/Zoo	In the past 7 days, did you/your child have any contact with a pet store, petting zoo, ranch, or farm where there were animals present?	[In the 7 days prior to illness] Did the patient visit or live on a farm? Did the patient visit a live animal exhibit?
Raw Dairy Consumption	In the past 7 days, did you/your child eat unpasteurized or raw milk from any animal?	Consumed any raw or unpasteurized milk or dairy products [in the 7 days prior to illness]?
Municipal Water Consumption	Does most of the water for you/your child's home come from a utility, also known as a municipal water or public water system?	What is the source of drinking water at work/school? (selected municipal)
Well Water Consumption	Does most of the water for you/your child's home come from an individual or private well?	What is the source of drinking water at work/school? (selected private or community well)
Natural Water Recreation	In the past 7 days, did you/your child swim, wade in, or enter an ocean, lake, pond, river, stream, or natural spring?	Was there recreational water exposure in the 7 days prior to illness? (selected hot spring, freshwater, ocean)
Treated Water Recreation	In the past 7 days, did you/your child swim, wade in, or enter a	Was there recreational water exposure in the 7 days prior to

	pool, hot tub, spa, fountain, or water park with treated water, such as chlorinated water?	illness? (selected hot tub, splash pad, water park, or swimming pool)
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Supplemental Table 2: Test types used to identify *Cryptosporidium* species in Tennessee, 2018–2023.^{a,b}

	<i>C. Parvum</i> (n=183)	<i>C. Hominis</i> (n=60)	<i>C. Felis</i> (n=8)	<i>C. Ubiquitum</i> (n=5)	All Cases (n=1174)
Polymerase Chain Reaction	151 (83%)	54 (90%)	6 (75%)	5 (100%)	761 (65%)
Enzyme Immunoassay	9 (5%)	4 (7%)	2 (25%)	0 (0%)	180 (15%)
Rapid Antigen	10 (5%)	2 (3%)	0 (0%)	0 (0%)	102 (9%)
Ova and Parasite Microscopy	0 (0%)	0 (0%)	0 (0%)	0 (0%)	28 (2%)
Acid Fast Staining	1 (>0%)	1 (2%)	0 (0%)	0 (0%)	6 (>0%)
Direct Fluorescent Antibody	1 (>0%)	0 (0%)	0 (0%)	0 (0%)	5 (>0%)
Other Tests	11 (6%)	1 (2%)	0 (0%)	0 (0%)	70 (6%)

^a Multiple test types could be used to diagnose a single *Cryptosporidium* infection.

^b Among 1,174 total cryptosporidiosis cases, 47 did not have test type available. Of these 45 had been laboratory-confirmed and test type was missing. Of these 45, 6 were *C. parvum* 1 was *C. hominis*, and 1 was *C. hominis*. Two were not laboratory-confirmed cases.

Supplemental Table 3. Characteristics of cryptosporidiosis cases, by availability of *Cryptosporidium* species and genotype, Tennessee, 2018–2023^a

	All Cases (n=1174) (%)	Species and Genotype Available (n=214) (%)	Species or Genotype Not Available (n=960) (%)
Demographic Factors			
Sex			
Male	532 (45%)	105 (49%)	427 (44%)
Female	642 (55%)	109 (51%)	533 (56%)
Age, years			
<18	374 (32%)	107 (50%)	267 (28%)
18–64	631 (54%)	99 (46%)	532 (55%)
≥65	169 (14%)	8 (4%)	161 (17%)
Race			
White	970 (83%)	180 (84%)	790 (82%)
Black	103 (9%)	18 (8%)	85 (9%)
Other	12 (1%)	1 (> 0%)	11 (1%)
Ethnicity			

Not Hispanic	1038 (88%)	187 (87%)	851 (89%)
Hispanic	31 (3%)	10 (5%)	21 (2%)
County Urban-Rural Class			
Large central metropolitan	171 (15%)	32 (15%)	139 (14%)
Large fringe metropolitan	193 (16%)	42 (20%)	151 (16%)
Medium metropolitan	302 (26%)	37 (17%)	265 (28%)
Small metropolitan	143 (12%)	18 (8%)	125 (13%)
Micropolitan	186 (16%)	30 (14%)	156 (16%)
Non-core	179 (15%)	55 (26%)	124 (13%)
International Travel			
Yes	72 (6%)	15 (7%)	57 (6%)
No	1102 (94%)	199 (93%)	903 (94%)
Contact with Person with Diarrheal Illness			
Yes	103 (9%)	32 (15%)	71 (7%)
No	727 (62%)	121 (57%)	606 (63%)
Animal Environment			
Pet Owner			
Yes	101 (9%)	31 (14%)	70 (7%)
No	766 (65%)	130 (61%)	636 (66%)
Zoo Visit			
Yes	47 (4%)	13 (6%)	34 (3%)
No	818 (70%)	148 (69%)	670 (70%)
Farm Visit			
Yes	231 (20%)	66 (31%)	165 (17%)
No	660 (56%)	101 (47%)	559 (58%)
Animal Contact			
Yes	638 (54%)	131 (61%)	507 (53%)
No	248 (21%)	37 (17%)	211 (22%)
Type of Animal Contact^b			
Goat	69 (6%)	23 (11%)	46 (5%)
Cow	208 (18%)	62 (29%)	146 (15%)
Sheep	24 (2%)	7 (3%)	17 (2%)
Dog	451 (38%)	89 (42%)	362 (38%)
Cat	201 (17%)	35 (16%)	166 (17%)
Chicken	121 (10%)	26 (12%)	95 (10%)
Turkey	9 (>0%)	1 (>0%)	8 (>0%)
Food and Water Consumption			
Fruits/Vegetables			
Yes	443 (38%)	75 (35%)	368 (38%)
No	319 (27%)	70 (33%)	249 (26%)
Shared Group Meal			

Yes	103 (9%)	17 (8%)	86 (9%)
No	718 (61%)	139 (65%)	579 (60%)
Restaurant Meals			
Yes	514 (44%)	94 (44%)	420 (44%)
No	248 (21%)	50 (23%)	198 (21%)
Raw Dairy			
Yes	15 (1%)	5 (2%)	10 (1%)
No	823 (70%)	150 (70%)	673 (70%)
Raw Cider			
Yes	12 (1%)	2 (1%)	10 (1%)
No	826 (70%)	155 (72%)	671 (70%)
Well Water			
Yes	96 (8%)	19 (9%)	77 (8%)
No	1078 (92%)	195 (91%)	883 (92%)
Recreational Water			
Yes	225 (19%)	53 (25%)	172 (18%)
No	678 (58%)	116 (54%)	562 (59%)
Recreational Water Type^b			
Ocean	32 (3%)	9 (4%)	23 (2%)
Freshwater	83 (7%)	19 (9%)	64 (7%)
Swimming Pool	103 (9%)	30 (14%)	73 (8%)
Splash Pad	23 (2%)	3 (1%)	20 (2%)
Hot Tub	12 (1%)	3 (1%)	9 (1%)
Water Park	27 (2%)	4 (2%)	23 (2%)
Hot Spring	0 (0%)	0 (0%)	0 (0%)

^aDifference in frequencies is from missing data.

^bResponses for animal contact and recreational water type could include multiple answers.

Supplemental Table 4: List of all *Cryptosporidium hominis* and *parvum* subtypes identified in Tennessee, 2018–2023.

	Number of cases (n=214)
<i>C. hominis</i>	
IaA14R3	1
IaA15R3	2
IaA18R6	1
IaA21R3	1
IaA23R3	6
IbA10G2	1
IbA10G2	4
IdA17	1
IdA17G1	10
IdA18	1
IdA26	2
IeA11G3T3	9
IfA12G1R5	15
IkA12	1
<i>C. parvum</i>	
IlaA13G2R1	1
IlaA14G1R1	1
IlaA15G2R1	80
IlaA15G2R2	15
IlaA15G2R3	1
IlaA16G2R1	1
IlaA16G3R1	4
IlaA16G3R2	1
IlaA17G2R1	7
IlaA17G2R2	11
IlaA17G2R3	15
IlaA17G2R4	1
IlaA17G3R1	6
IlaA17G4R1	1
IlaA18G2	1
IlaA18G3R1	1
IlaA19G2R2	1
IlcA5G3a	4
IlcA5G3b	1
IldA15G1	2
IldA17G1	1
IldA20G1	1
IldA22G1	1
Deer Mouse Subtype ^a	1

^aThis isolate was originally reported in 2018 as *C. parvum* deer mouse subtype. When reviewed with current taxonomy understanding in 2025, the isolate's sequence more closely resembles *C. species* deer mouse genotype than *C. parvum*. In the analysis, we categorized species based on the original testing result.