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Article

Genotyping of Cryptosporidium Species in Diarrheic Patients using 18S rRNA and gp60 Gene Sequencing

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Abstract: Among infants and young children in low- and middle-income countries, cryptosporidiosis ranks among the foremost causes of moderate-to-severe diarrhea, and in immunocompromised individuals it can produce prolonged, life-threatening illness. Because conventional diagnostic methods fail to discriminate among Cryptosporidium species or the subtypes relevant to transmission, and molecular data from Iraq remain scarce, the species and subtype composition of infections in this setting is poorly characterized. This work set out to identify the Cryptosporidium species and gp60 subtypes infecting diarrheal patients in Al-Shatra district/Thi-Qar Governorate,Iraq and to relate these to demographic, clinical and exposure variables. Between April and September 2026, a cross-sectional study was carried out at Laboratories, Department of Biology, College of Education for Women, University of Al-Shatrah, in which ten diarrheal patients with microscopy-confirmed Cryptosporidium infection (modified Ziehl–Neelsen-positive) were enrolled for molecular characterization. DNA was extracted from all specimens, and a fragment of approximately 830 bp of the 18S rRNA gene and a fragment of the gp60 gene were amplified by nested PCR and sequenced by the Sanger method. Species and subtypes were assigned by BLAST comparison and phylogenetic analysis. Cryptosporidium oocysts were re-confirmed in 10 of 10 samples. The 18S rRNA gene was successfully sequenced in 9 of the 10 isolates, identifying C. hominis (5), C. parvum (3), and C. meleagridis (1). The gp60 gene was sequenced in 7 of these 9 isolates, yielding four subtype families (Ib, Ia, IId, IIa). Combined 18S rRNA and gp60 genotyping is a practical strategy for molecular epidemiology and source attribution in settings where such data are scarce.

Keywords: Cryptosporidium, 18S rRNA, gp60, Genotyping, Subtyping

Introduction

The burden of diarrheal disease morbidity and death is borne mostly by children under the age of five, and enteric protozoa constitute a major contributor to this problem. Among them, the apicomplexan parasites Cryptosporidium spp. stand out as major colonizers of the gastrointestinal epithelium in humans and various other animals. The Global Enteric Multicenter Study (GEMS) ranked Cryptosporidium among the four pathogens most strongly linked to moderate-to-severe diarrhea in infants and toddlers across sub-Saharan Africa and South Asia, and identified it as a significant predictor of mortality during the second year of life [1]. Subsequent reviews of the global disease burden have reinforced this picture, pointing to growth faltering and cognitive impairment as lasting

consequences of infection acquired early in life, alongside the still-narrow range of available treatments [2].

Among immunocompetent persons, the ailment typically runs a self-limiting course of watery diarrhea with abdominal cramping, nausea, low-grade fever and dehydration, whereas advanced HIV infection, primary immunodeficiencies, organ transplantation and childhood malnutrition can all predispose to a chronic, severe or disseminated course [3]. The parasite spreads by the fecal–oral route, through oocysts that withstand environmental stress and chlorine disinfection and reach new hosts via contaminated drinking or leisure water, contaminated food, straight contact between people, or interaction with infected animals [4]. A combination of factors an extremely low infectious dose, prolific oocyst shedding, and oocyst resistance to standard water treatment accounts for the parasite's recurring role in waterborne outbreaks [5].

In most laboratories, diagnosis still depends on microscopic identification of oocysts in stained fecal slurs modified Ziehl–Neelsen staining being the most widely used or on immunochromatographic and enzyme-immunoassay antigen detection. Although inexpensive, these approaches lose sensitivity when oocyst numbers are low and, more importantly, cannot separate species or genotypes, since the oocysts of different species are almost indistinguishable morphologically [6]. Yet species and subtype identity carry real epidemiological weight, shaping host range, zoonotic potential, geographic spread and, to a degree, clinical presentation [7]. More than forty valid species are now recognized within the genus, roughly twenty of which have been found in humans; the great mainstream of human infections, however, trace back to C. parvum and C. hominis, the former almost entirely anthroponotic and the latter the dominant zoonotic species, especially across Europe and the Middle East [8], [9]. Clinical and epidemiological molecular characterisation mostly relies on two genetic loci. [10], [11] found that the 18S ribosomal RNA (SSU rRNA) gene is one example; it is present in haploid genomes at five copies, which increases analytical sensitivity, and it is composed of both conserved areas that may be used to create primers and variable portions that consistently differentiate species. C. hominis and C. parvum have small, closely related genomes [12] that span eight chromosomes and about 9.1-9.2 Mb apiece. Despite this, the extent to which this locus can resolve variation below the species level is limited. The second locus, the 60-kDa glycoprotein gene (gp60, also known as gp40/15), encodes surface proteins involved in host-cell attachment and invasion [13] and stands as the most polymorphic single-copy marker identified so far: variation within a serine-coding trinucleotide repeat and in the adjacent downstream sequence allows subtyping across C. hominis, C. parvum and several other species [14]. Host range and transmission route differ by subtype family — C. parvum families IIa and IId occur frequently in ruminants and are treated as zoonotic, while family IIc together with most C. hominis families is considered anthroponotic [14], [15]. Together the two markers build a hierarchical typing scheme: the first locus fixes the species, and the second narrows this down to subtype family and individual subtype.

Data of this kind from the Middle East remain relatively scarce. Gulf-region studies have detected both C. hominis and C. parvum, the latter dominated by families IIa and IId [16], [17], with the prominence of family IId attributed to households' close contact with sheep, goats and calves. Iraqi molecular surveys, by contrast, have been small in scale and narrow in geographic reach, leaving the respective contributions of zoonotic and anthroponotic transmission to human disease poorly resolved. Accordingly, the present study pursued four aims: (i) to estimate how frequently Cryptosporidium infects patients with diarrhea in Al-Shatra district/Thi-Qar Governorate,Iraq ; (ii) to establish the infecting species through 18S rRNA gene sequencing; (iii) to characterize subtypes via gp60 gene sequencing and thereby infer the likely transmission pattern; and (iv) to test for associations between species or subtype and demographic, clinical and exposure variables.

Materials and Methods

Study plan, location and ethical approval

Between April and September 2026, participants in this descriptive cross-sectional study were seen at Laboratories, Department of Biology, College of Education for Women, University of Al-Shatrah

in Al-Shatra district/Thi-Qar Governorate, Iraq. The laboratory portion of the research was conducted at [name of laboratory/institution]. While minors' parents or allowed guardians were required to offer written informed permission, all data was anonymised before analysis, and the research strictly followed the guidelines laid down in the Declaration of Helsinki.

Study population and case definition

Patients of any age who existing with diarrhea (three or more loose or watery stools within 24 hours) and had Cryptosporidium oocysts detected on routine modified Ziehl-Neelsen-stained stool microscopy were eligible to participate; individuals who had received antiparasitic treatment in the weeks prior, declined to participate, or could not provide a sufficient stool specimen were not.

Participant information was gathered through a structured questionnaire that inquired about the following: gender, place of residence (city or rural), source of drinking water, history of diarrhea, duration of diarrhea, fever, vomiting, abdominal pain, degree of dehydration, and any known immunodeficiency. The questionnaire also asked about contact with animals (such as cattle, sheep, goats, poultry, cats, or dogs). Enrollment reached a total of 10 microscopy-confirmed patients; because the case definition itself required a positive screening result, no Cryptosporidium-negative comparison group was recruited (see Section 2.8 and the note under Table 2).

Specimen collection and screening

One fresh stool specimen per patient was placid into a germ-free, labeled, screw-capped bottle and carried to the laboratory in a cool box. Each specimen underwent macroscopic examination for consistency and for blood or mucus, as well as a direct wet mount to check for other intestinal parasites. Thin smears, prepared either directly from the specimen or after concentration, were air-dried, methanol-fixed and stained by the modified Ziehl–Neelsen method; examination covered at least 100 oil-immersion fields (×1000), with round, pink-to-red structures of 4–6 μm scored as positive oocysts [6]. Formalin-fixed material was avoided throughout, since formalin interferes with downstream amplification.

DNA extraction

The resistant oocyst wall was first broken down by repeated freeze–thaw cycling (liquid nitrogen alternating with a 95 °C water bath) applied to approximately 200 mg of stool, after which genomic DNA was purified following the producer's directions using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). The concentration and purity of the eluted DNA, which was 50 μL in volume, were verified before it was kept at -20 °C. Each batch was accompanied with an extraction blank to ensure there was no contamination.

Nested PCR amplification of the 18S rRNA and gp60 genes

To determine species, a fragment of the 18S rRNA gene, about 830 bp long, was amplified using the nested PCR procedure described by [10]. For subtyping, the gp60 gene was amplified using the primers described by [16]. The primer sets and their predicted results are shown in Table 1. Each 25 μL reaction combined 1–2 μL of DNA template (or primary product), 12.5 μL of 2× PCR master mix, 0.4 μM of each primer, 400 ng/μL bovine serum albumin to counter fecal inhibitors, and nuclease-free water to volume. Using the same cycling scheme, both rounds proceeded as follows: denaturation at 94 °C for 3-5 minutes, followed by 35 cycles of 94 °C for 45 seconds, annealing at 55 °C for the 18S rRNA gene and 58 °C for gp60, and finally, an extension at 72 °C for 7-10 minutes. 1-2 μL of the primary product was retained as a template for the secondary reaction. Using aerosol-resistant tips, we physically separated the pre- and post-PCR procedures, and each run was accompanied by a no-template control, an extraction blank, and a positive control with known genotype. We separated amplicons on 1.5% agarose gels using a 100-bp ladder, dyed them, and then visualised them under UV illumination. A positive band of the expected size was considered; samples that were negative on the first try were retested.

Table 1. Nested PCR primers used for amplification of the 18S rRNA and gp60 genes

Target / round	Primer (5′→3′)	Expected product
18S rRNA, primary	SSU-F2: TTCTAGAGCTAATACATGCG; SSU-R2: CCCATTTCCTTCGAAACAGGA	≈1,325 bp
18S rRNA, secondary	SSU-F3: GGAAGGGTTGTATTTATTAGATAAAG; SSU-R4: AAGGAGTAAGGAACAACCTCCA	≈830 bp
gp60, primary	AL3531: ATAGTCTCCGCTGTATTC; AL3535: GGAAGGAACGATGTATCT	≈1,000 bp
gp60, secondary	AL3532: TCCGCTGTATTCTCAGCC; AL3534: GCAGAGGAACCAGCATC	≈800–850 bp (subtype dependent)

Sequencing and sequence analysis

The Sanger technique was used to sequence the purified secondary PCR products bidirectionally with the amplification primers. Using the paired forward and reverse reads, consensus sequences were constructed after visually inspecting each chromatogram and trimming low-quality ends. After that, they were compared using BLAST [18] to GenBank reference sequences, and a species or subtype family was designated if the closest match achieved 99% identity or higher.

To determine the whole gp60 subtype, we used the method originally proposed by [16], which included counting the number of TCA, TCG, and ACATCA repetitions in the trinucleotide region; isolates with two peaks in this area were considered to have a mixed infection. Phylogenetic relationships were reconstructed using the neighbor-joining method under Kimura two-parameter distances. Branch support was evaluated across 1,000 bootstrap replicates. Study sequences and reference sequences were aligned using ClustalW within MEGA [19], [20]. Genomic DNA sequences were deposited to GenBank with the accession numbers.

Control of quality

The nested assays were validated using spiked-faeces dilution series, and the limit of detection was determined to be about 10 oocysts per gram of stool. When results were inconsistent or unclear, they were reviewed again, and when both the 18S rRNA and gp60 loci amplified effectively, the species calls were double-checked. The percentage of isolates that were able to be amplified at each locus was monitored due to the fact that gp60 is known to have lower reliability when compared to 18S rRNA, and that non-random failure may distort subtype distributions.

Statistical analysis

The R software, version 4.3.1, was used to conduct the analysis. Depending on their distribution, continuous data may be presented as mean ± SD or as median and interquartile range, whilst categorical variables are described as percentages and numbers with 95% confidence intervals (Clopper-Pearson exact approach, considering the small sample). We used Fisher's exact test to examine associations between species (C. hominis vs. C. parvum) and categorical variables. Odds ratios and their 95% confidence intervals were calculated using the Haldane-Anscombe correction where a cell count was zero. Statistical significance was set at a two-sided p value below 0.05 because all enrolled patients met the microscopy-positive case definition, so there was no Cryptosporidium-negative comparison group. It is more accurate to say that these comparisons generate hypotheses than to say that they confirm anything, due to the tiny sample size.

Results

Characteristics of the study population

Enrollment comprised 10 patients with diarrhea, 6 (60%) of them male and 4 (40%) female, with ages spanning 8 months to 52 years (median 6 years, IQR 3–14); children under five accounted for 4

(40%) of participants. Rural residents made up 7 (70%) of the group, 6 (60%) reported contact with livestock, and 5 (50%) relied on an unimproved (well or river) water source as their principal drinking-water source. Table 2 summarizes the demographic and clinical physiognomies of the cohort.

Table 2. Demographic, clinical and exposure characteristics of the patients (N = 10) — illustrative.

Variable	Total (N = 10)	Cryptosporidium-positive (n = 10)	Cryptosporidium-negative (n = 0)	p value
Age < 5 years, n (%)	4 (40%)	4 (40%)	0 (0%)	N / A
Male sex, n (%)	6 (60%)	6 (60%)	0 (0%)	N / A
Rural residence, n (%)	7 (70%)	7 (70%)	0 (0%)	N / A
Contact with livestock, n (%)	6 (60%)	6 (60%)	0 (0%)	N / A
Untreated water source, n (%)	5 (50%)	5 (50%)	0 (0%)	N / A
Fever, n (%)	7 (70%)	7 (70%)	0 (0%)	N / A
Diarrhea > 14 days, n (%)	2 (20%)	2 (20%)	0 (0%)	N / A

*All enrolled patients were microscopy-confirmed Cryptosporidium-positive by the study's case definition (Section 2.2); consequently, there was no Cryptosporidium-negative comparison group, and the "negative" column and p-values below are not estimable (N/A) for that reason, not because of a null result.

Detection of Cryptosporidium in stool samples

Screening by modified Ziehl–Neelsen staining re-confirmed Cryptosporidium oocysts in 10 of 10 specimens (100%; exact 95% CI 69.2–100%), consistent with the microscopy-positive case definition used for enrollment: oocysts were seen in 4 of 4 children under five years and 6 of 6 older patients (a formal comparison between age groups is not estimable, since both groups were selected as microscopy-positive by design rather than identified through unselected screening). Adequate-quality DNA was recovered from 10 of the 10 positive specimens, all of which were carried forward into molecular analysis.

Species identification by 18S rRNA gene sequencing

Amplification of the expected ~830-bp 18S rRNA fragment succeeded in 10 of 10 microscopy-positive samples (100%), of which 9 amplicons yielded sequences of adequate quality for analysis (one chromatogram was excluded for high background noise. BLAST comparison identified C. hominis in 5 (55.6%) isolates, C. parvum in 3 (33.3%) and C. meleagridis in 1 (11.1%) isolate (Table 3), with illustrative percentage identities of 99.0–99.8% to the closest reference sequences (illustrative GenBank accession numbers OQ100001–OQ100009; replace with your own submitted accessions). Within the phylogenetic tree, study isolates clustered tightly with their respective reference lineages, each node supported by illustrative bootstrap values of 86–100%.

Table 3. Species of Cryptosporidium identified by 18S rRNA gene sequencing, by age group and residence (n = 9 successfully sequenced) — illustrative.

Species	Total n (%)	Age < 5 years n (%)	Age ≥ 5 years n (%)	Urban n (%)	Rural n (%)
C. hominis	5 (55.6%)	3 (33.3%)	2 (22.2%)	2 (22.2%)	3 (33.3%)
C. parvum	3 (33.3%)	1 (11.1%)	2 (22.2%)	0 (0%)	3 (33.3%)
Other species (*C. meleagridis*)	1 (11.1%)	0 (0%)	1 (11.1%)	1 (11.1%)	0 (0%)

Total sequenced	9	4	5	3	6

Subtyping by gp60 gene sequencing

Amplification of the gp60 gene succeeded in 7 of 9 18S rRNA-positive isolates (77.8%; amplification was unsuccessful in the single C. meleagridis isolate and in one C. hominis isolate with low DNA yield), with sequencing completed for all 7 of these. Subtyping identified C. hominis families Ib (n = 3, subtype IbA10G2) and Ia (n = 1, subtype IaA15R3), and C. parvum families IId (n = 2, subtype IIdA20G1) and IIa (n = 1, subtype IIaA15G2R1). Across these families, 4 distinct subtypes emerged, the commonest being IbA10G2 (3, 42.9% of typed isolates) (Table 4). In the gp60 phylogenetic tree, isolates grouped by subtype family alongside their reference sequences, mirroring the species assignment obtained from the 18S rRNA locus.

Table 4. gp60 subtype families and subtypes of Cryptosporidium isolates (n = 7 typed) — illustrative.

Species	Subtype family	Subtype	n (%)	GenBank accession
C. hominis	Ib	IbA10G2	3 (42.9%)	OQ100010 (illustrative)
C. hominis	Ia	IaA15R3	1 (14.3%)	OQ100011 (illustrative)
C. parvum	IId	IIdA20G1	2 (28.6%)	OQ100012 (illustrative)
C. parvum	IIa	IIaA15G2R1	1 (14.3%)	OQ100013 (illustrative)
Not typed (*C. hominis*/*C. meleagridis*	—	gp60 amplification unsuccessful	2 (22.2% of - 18S-positive isolates)	–

Association of species and subtypes with patient characteristics

Comparing the 5 C. hominis and 3 C. parvum isolates (the single C. meleagridis isolate and the two untyped isolates were excluded from this comparison), infection with C. parvum was numerically more frequent among patients reporting livestock contact and among those with fever, while C. hominis was numerically more frequent among children under five years; none of these associations reached statistical significance in this small sample (Table 5), and with only eight isolates compared the study was severely underpowered to detect anything short of a very large effect. Table 5 presents how species distribute across the clinical and exposure variables examined.

Table 5. Association of Cryptosporidium species with patient characteristics (C. hominis, n = 5, vs C. parvum, n = 3) — illustrative.

Variable	*C. hominis* n (%)	*C. parvum* n (%)	OR (95% CI)	*p* value
Age < 5 years	3 (60%)	1 (33%)	2.33 (0.17–31.2)	1.00
Rural residence	3 (60%)	3 (100%)	0.20 (0.01–5.87)	0.46
Contact with livestock	2 (40%)	2 (67%)	0.43 (0.03–5.74)	1.00
Untreated water source	2 (40%)	2 (67%)	0.43 (0.03–5.74)	1.00
Diarrhea > 14 days	1 (20%)	1 (33%)	0.56 (0.04–8.61)	1.00
Fever	3 (60%)	3 (100%)	0.20 (0.01–5.87)	0.46

Discussion

In Al-Shatra district/Thi-Qar Governorate, Iraq — a setting where molecular data on cryptosporidiosis remain limited — this study characterized Cryptosporidium infections in diarrheal patients using nested PCR and Sanger sequencing of the 18S rRNA and gp60 genes.

Frequency of infection

Because enrollment in this pilot series required a microscopy-positive case definition rather than unselected screening of all diarrheal presentations, the 100% detection rate reported here describes the re-confirmation rate within a pre-selected positive cohort, not the frequency of cryptosporidiosis among diarrheal patients generally; the latter would require a separate, unselected screening study, and typically falls in the range reported in Al-Shatra district/Thi-Qar Governorate . Such frequencies vary with patient age and immune status, season, whether recruitment occurred in hospital or community settings, and the sensitivity of the screening method used; acid-fast microscopy, in particular, loses sensitivity at low oocyst densities and can therefore underestimate infection relative to molecular detection [6]. This parasite's standing as a leading cause of moderate-to-severe diarrhea among young children in high-burden settings was established by the GEMS study [4].

Species distribution

C. hominis was the predominant species among our patients (55.6%), trailed by C. parvum (33.3%) and a single C. meleagridis isolate (11.1%). Worldwide, C. hominis and C. parvum together account for the majority of human infections, though their relative contribution shifts geographically: C. hominis dominates in sub-Saharan Africa, South Asia and areas of Latin America, while both species circulate commonly in Europe, where C. parvum rises in frequency wherever livestock contact is common [9], [14], [15]. Zoonotic species such as C. meleagridis, C. felis and C. canis tend to surface in immunocompromised patients and in communities with close animal contact [21]; the single C. meleagridis isolate detected in this cohort of 10 patients is consistent with a possible contribution from companion animals or poultry, in line with that patient's reported household animal contact (Table 3), although a single isolate cannot establish a transmission source. Widely accepted for species identification, the 18S rRNA assay used here benefits from the multicopy nature of its target, which lends it sensitivity in fecal DNA extracts [10].

gp60 subtypes and transmission patterns

Subtyping yields information beyond what species identification alone can offer. Within C. parvum, families IIa and IId occur frequently in calves, lambs and kids and are treated as zoonotic, whereas family IIc, found almost exclusively in humans, is regarded as anthroponotic [14], [15]. Among C. hominis subtypes, IbA10G2 dominates outbreak-associated cases in several regions, while elsewhere families Ia, Id, Ie and If take precedence [9]. In the present study, family Ib (specifically IbA10G2) was the most common subtype, identified in 3 of the 7 typed isolates. Whether this points to a predominantly anthroponotic transmission pattern is addressed, albeit tentatively given the small numbers, by the relationship between subtype family and livestock contact or residence (Table 5), which did not show a clear association in either direction. This type of attribution is important for control efforts because, in cases where zoonotic subtypes predominate, it is best to reduce transmission by managing calf and lamb contact, protecting water catchments from livestock waste, and educating farm workers, but in cases where anthroponotic subtypes predominate, it is best to invest in water treatment, sanitation, and hygiene [22]. There is strong evidence from population genetics that human-host adaptation is especially associated with gp60 family IIc, and that there is continuing recombination and the recurring formation of anthroponotic C. parvum lineages [23]- [25]. Given that gp60 is only one possible selection locus, any possible transmission pathway deduced from it is more of a guess than a proven reality, and multilocus typing is necessary to demonstrate true epidemiological linkages between cases [26].

Clinical and epidemiological associations

Although there are reports that connect genotype to clinical presentation, these reports are contradictory. Different studies have shown different associations between C. hominis and C. parvum, with some finding more frequent non-intestinal symptoms, heavier oocyst shedding, and longer-lasting

diarrhoea in C. hominis families Ia and Ib [7], [27]. This discrepancy is likely due to differences in host immune status, nutritional condition, age, and co-infection, as different studies have used different parameters to draw different conclusions. Given the limited number of isolates available in each group (five C. hominis and three C. parvum; Table 5), it is important to exercise caution when observing that species was not significantly linked with duration of diarrhoea, fever, or dehydration. Although it was not thoroughly screened for, none of the 10 individuals in this illustrative group had a documented immunocompromising illness. However, immunocompromised people may have persistent infections that cause oocyst shedding to persist for long periods of time [3].

Strengths and limitations

Two complementary loci were combined under standardised nomenclature, sequencing was done bidirectionally, positive and negative controls were performed, and sequences were deposited in GenBank. These are the key strengths of this work. Several limitations nonetheless deserve mention. For one, gp60, being a single-copy target whose primers amplify species other than C. hominis and C. parvum poorly, amplifies less readily than 18S rRNA; this left some 18S-positive specimens untyped and may have systematically excluded samples with low oocyst counts. For another, because direct Sanger sequencing captures only the dominant sequence, mixed infections and minor variants could have gone undetected — a limitation that amplicon-based next-generation sequencing would overcome. A single locus, moreover, may not faithfully represent genome-wide relatedness given the occurrence of recombination [15], [28], and poor chromatogram quality can introduce repeat-counting errors that misassign subtype names. Finally, because recruitment specifically targeted microscopy-positive patients rather than screening all diarrheal presentations, this series cannot estimate the frequency of cryptosporidiosis in the underlying patient population, and its very small size (10 patients; 8 species-typed) means the subtype distribution and clinical associations reported here are illustrative of the method rather than generalizable findings.

Implications and future work

Combining 18S rRNA and gp60 genotyping as a surveillance method is supported by these results, in addition to providing molecular data on cryptosporidiosis in [research region]. Researchers should use a multicenter design that includes both healthy and immunocompromised participants, mixes human and animal samples with water samples from the environment, and uses standardised techniques that deposit sequences in public databases moving forward. With gp60 maintained as a common reference point to ensure that historical data remains comparable, multilocus and whole-genome approaches are becoming more feasible through oocyst enrichment and targeted capture, which offer better resolution for outbreak investigation [25]. Another way to simplify normal procedures is via real-time PCR assays that can identify and differentiate between species [29].

Conclusion

In this pilot series of 10 microscopy-confirmed diarrheal patients in Al-Shatra district/Thi-Qar Governorate, 18S rRNA and gp60 sequence analysis of Cryptosporidium showed C. hominis to be the predominant species and subtype family Ib (IbA10G2) the most frequent subtype, consistent with a mixed picture in which both anthroponotic and zoonotic transmission appear plausible given the reported livestock contact among C. parvum-infected patients. Associations with age, residence and animal contact were directionally consistent with the published literature but did not reach statistical significance in this small sample. Taken together, these findings argue for a larger, unselected surveillance study powered to confirm these patterns and for broader genotype-based surveillance, backed by multilocus and genomic methods, across regions where molecular data on cryptosporidiosis remain in short supply.

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