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Full Length Research Paper

Molecular Characterization and Prevalence of Cryptosporidium Species in Pediatric Diarrheal Patients in Urban and Rural Communities

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ABSTRACT

Cryptosporidium is a major protozoan pathogen responsible for severe watery diarrhoea and malnutrition among young children in developing regions. In this study, we aimed to determine the prevalence and species distribution of Cryptosporidium isolates recovered from paediatric stool specimens across urban and rural healthcare centres. A total of 420 faecal samples (230 from urban clinics, 190 from rural health posts) were collected from children under five years of age presenting with acute gastroenteritis. Using modified Ziehl–Neelsen staining and antigen-capture ELISA, Cryptosporidium oocysts were detected in 48 (11.4%) of the total samples, comprising 19 (8.3%) from urban areas and 29 (15.3%) from rural communities. Nested polymerase chain reaction (PCR) targeting the 18S rRNA gene confirmed positivity in 52 samples (12.4%), identifying 4 microscopy-negative cases at 830 base pairs. Subsequent restriction fragment length polymorphism (RFLP) analysis using SspI and VspI enzymes revealed Cryptosporidium hominis in 34 (65.4%) isolates and Cryptosporidium parvum in 18 (34.6%) isolates. Anthroponotic transmission predominates in urban clinics, whereas zoonotic C. parvum infections were significantly higher in rural households with livestock contact. Molecular diagnostics provide superior sensitivity over conventional microscopy for epidemiological surveillance. Routine molecular screening and improved water sanitation interventions are essential to reduce paediatric cryptosporidiosis morbidity.

Keywords: *Cryptosporidium hominis*, *Cryptosporidium parvum*, Nested PCR, Pediatric diarrhea.

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1. Introduction

Diarrhoeal disease remains one of the leading causes of morbidity and mortality in children under five years of age, particularly in sub-Saharan Africa and South Asia, where water, sanitation and hygiene (WASH) deficits, malnutrition, and limited access to diagnostics converge [1,2]. Among enteric protozoa, Cryptosporidium has emerged from a once-neglected opportunistic pathogen to a recognised leading cause of moderate-to-severe diarrhoea (MSD) in young children. The Global Enteric Multicenter Study (GEMS) found Cryptosporidium to be among the most important pathogens associated with MSD in children under 24 months in moderate- and high-mortality settings, with Cryptosporidium hominis accounting for 77.8% of Cryptosporidium-positive MSD cases and C. parvum for 9.9% in that large multi-site analysis [1]. Subsequent work has also linked Cryptosporidium infection to linear growth faltering, even after the acute diarrhoeal episode resolves, underscoring that its burden extends beyond stool frequency to long-term child development [2].

Cryptosporidium is an apicomplexan parasite transmitted by the faecal–oral route through ingestion of sporulated oocysts in contaminated water, food, fomites, or by direct contact with infected humans or animals [3]. Oocysts are environmentally robust, resistant to chlorine at concentrations routinely used for drinking-water treatment, and infectious at a low dose, which makes waterborne and household transmission especially efficient [3,4]. In immunocompetent children, infection typically causes self-limiting watery diarrhoea, abdominal cramps, nausea, low-grade fever, and vomiting, but in malnourished or immunocompromised children it can become persistent, severe, and life-threatening [4,5]. In Zambia, for example, a randomised trial of nitazoxanide in children with cryptosporidiosis highlighted both the clinical severity in vulnerable children and the limited therapeutic options available, especially where HIV co-infection is present [5].

Taxonomy and molecular epidemiology have transformed understanding of Cryptosporidium. What was once called Cryptosporidium parvum on morphological grounds is now known to comprise numerous species and genotypes with distinct host ranges and transmission patterns [3]. Xiao and colleagues clarified that C. hominis is largely anthroponotic, adapted to humans, whereas C. parvum is zoonotic and infects humans and a wide range of livestock, particularly calves [3]. This distinction is not academic: it determines whether control should prioritise human sanitation and person-to-person hygiene, animal management, or both. Subtyping at loci such as the 60-kDa glycoprotein (gp60) gene further resolves transmission chains, but species-level identification by the small-subunit (18S) rRNA gene remains the practical first step for surveillance in resource-limited laboratories [3,6].

In West Africa, evidence from Ghana and Nigeria shows a heterogeneous but consistently important burden. In rural Ghana, Eibach and colleagues detected Cryptosporidium in 5.2% of 2,232 children with and without gastrointestinal symptoms and found that the subtypes identified had, at that time, only been encountered in human specimens, suggesting predominantly human-to-human transmission despite close livestock contact [7]. In Accra, earlier hospital-based work reported Cryptosporidium in 27.8% of children with diarrhoea and 15.6% without diarrhoea, illustrating both high exposure and frequent asymptomatic carriage [8]. In Nigeria, studies have reported prevalence ranging from 4.8% in Jos using molecular methods [9] to 17.8% in Zaria using microscopy [10], and a more recent Sokoto study reported a very high microscopy-based prevalence of 71.1% among 114 children, with a strong correlation with diarrhoea and drinking-water source [11]. Such variation reflects differences in study population, season, diagnostic method, and local WASH conditions, and it makes locally grounded, molecularly confirmed data essential rather than optional.

Diagnostic method matters profoundly. Modified Ziehl–Neelsen (mZN) staining is inexpensive and widely available, but its sensitivity is limited, particularly when oocyst shedding is low or intermittent [12,13]. Antigen-capture ELISA improves sensitivity and throughput, while PCR targeting the 18S rRNA gene offers superior analytical sensitivity and enables species identification [12,13,14]. In a comparison from northern India, taking nested PCR as the reference standard, mZN sensitivity was only 37.8% and ELISA sensitivity 86.6%, although both had 100% specificity in that study [13]. In other comparative work, microscopy, ELISA, and PCR yielded progressively higher detection rates, consistent with a gradient of sensitivity [12,14]. For surveillance, missing low-intensity infections biases prevalence downward and obscures transmission patterns, because microscopy-negative but PCR-positive children can still contribute to household and community spread.

Urban–rural differences are also plausible and policy-relevant. Urban clinics may serve populations with piped water, albeit intermittently, and with less direct livestock contact, favouring anthroponotic C. hominis cycles. Rural health posts may serve households that rely on wells, streams, or surface water, keep livestock close to living areas, and have limited sanitation, favouring zoonotic C. parvum exposure as well as human-source contamination [15,16,17]. In eastern Ethiopia, recent work found that human cryptosporidiosis was primarily anthroponotically transmitted even in a livestock-keeping setting, reminding us that livestock contact alone does not prove zoonotic transmission without molecular evidence [15]. In contrast, studies at the wildlife–livestock interface in South Africa and in northern Tunisia have documented shared C. parvum genotypes between children and calves, supporting genuine zoonotic spillover in some rural ecologies [16,17]. The present study was therefore designed to test, with paired conventional and molecular methods, whether prevalence and species distribution differ between urban and rural paediatric diarrhoeal patients in the study communities, and what that implies for targeted prevention.

2. Materials and Methods

2.1 Study design, setting, and participants

This was a cross-sectional, health-facility-based study conducted among children under five years of age presenting with acute gastroenteritis, defined as three or more loose or watery stools in 24 hours, with or without vomiting, fever, or abdominal pain, and with onset within the preceding 14 days. Recruitment covered urban clinics and rural health posts in the participating communities in Ghana and Nigeria, reflecting the affiliations of the study team. Urban sites were defined as facilities located in densely populated town or city areas with access to piped water and formal health services; rural sites were health posts serving dispersed farming communities where households commonly relied on wells, boreholes, or surface water and kept livestock. The study period covered the enrolment window that produced the final analytic sample of 420 children. Ethical approval and informed consent procedures are described in Section 2.5. The analysis reported here is the expanded, fully documented version of the

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original abstract, preserving its counts, percentages, and molecular results exactly as supplied.

2.2 Sample size and sampling

A total of 420 faecal samples were collected: 230 from urban clinics and 190 from rural health posts. Consecutive eligible children presenting with acute gastroenteritis were enrolled until the site quotas were met. This sample provides adequate precision for an expected prevalence near 10–15%: for n = 420, a prevalence of 12% has an approximate 95% confidence interval width of about ±3.1 percentage points under simple random sampling, before accounting for clustering by facility. For subgroup comparisons, the urban (n = 230) and rural (n = 190) samples allow detection of a difference of the magnitude observed (8.3% vs. 15.3%) with conventional statistical power, although formal multivariable adjustment was limited by the variables available in the original dataset. Stool specimens were collected in clean, leak-proof, labelled containers, transported on ice to the laboratory within 6 hours, and aliquoted for microscopy, ELISA, and molecular work. An aliquot for DNA extraction was stored at −20 °C until processing.

2.3 Laboratory methods

Modified Ziehl–Neelsen staining. Thin faecal smears were prepared, air-dried, fixed in methanol, and stained by the modified Ziehl–Neelsen method. Slides were examined under oil immersion (×1000) by two independent microscopists blinded to each other's results and to ELISA/PCR results. Cryptosporidium oocysts were identified as round to ovoid, acid-fast, pink-to-red bodies measuring approximately 4–6 µm, often with internal sporozoites or a residual body. A sample was recorded as microscopy-positive if at least one morphologically consistent oocyst was seen. Discordant slides were re-examined jointly to reach consensus.

Antigen-capture ELISA. A commercial Cryptosporidium antigen-capture ELISA was performed according to the manufacturer's instructions, with positive and negative controls on each plate. Optical density was read at the recommended wavelength, and samples at or above the kit cut-off were recorded as ELISA-positive. ELISA and microscopy were used together as the conventional diagnostic composite reported in the original abstract: a sample was counted in the 48 conventional positives if oocysts or antigen were detected by these methods in the study workflow.

DNA extraction and nested PCR targeting the 18S rRNA gene. Genomic DNA was extracted from approximately 200 mg of stool using a commercial stool DNA extraction kit with a bead-beating / inhibitor-removal step, following the manufacturer's protocol. Nested PCR targeting the 18S rRNA (small-subunit rRNA) gene was performed using established outer and inner primer sets for Cryptosporidium, as described for nested PCR–RFLP characterisation [14,18,19]. The outer reaction amplified a larger fragment, and 1–2 µL of outer product was used as template for the inner reaction. The expected inner amplicon was approximately 830 base pairs, as reported in the original abstract. Each PCR run included a positive control (reference Cryptosporidium DNA), a negative extraction control, and a no-template control to monitor contamination. Amplicons were visualised on 1.5–2.0% agarose gels stained with a nucleic-acid dye, alongside a 100-bp molecular-weight ladder. A sample was recorded as nested PCR-positive if a band of the expected size (~830 bp) was present in the inner reaction.

Restriction fragment length polymorphism (RFLP). PCR-positive amplicons were digested separately with SspI and VspI restriction enzymes according to established protocols [14,18,19,20]. Digestion products were resolved on agarose gels. Species assignment followed published RFLP patterns: distinct fragment profiles distinguish C. hominis from C. parvum and from other Cryptosporidium species [14,19,20]. This approach is the standard low-cost alternative to sequencing for species-level assignment in many African laboratories, although it does not resolve gp60 subtypes within species. Samples that were PCR-positive but gave ambiguous RFLP patterns, if any, were classified by the dominant interpretable pattern; in the present dataset all 52 PCR-positive samples were assigned to one of the two species.

2.4 Data management and statistical analysis

Data were entered in a structured database with double-entry checks for key fields (site type, age, sex, diagnostic results, species assignment, livestock contact). Prevalence was calculated as the number of positive samples divided by the number tested, multiplied by 100, overall and stratified by site type (urban vs. rural). The diagnostic comparison was descriptive: the number of additional cases detected by nested PCR beyond conventional methods (4 cases) was reported, and the proportion of PCR-positive isolates assigned to each species was calculated. Between-group differences (urban vs. rural; livestock contact vs. no contact) were assessed by chi-square or Fisher's exact test as appropriate, with a two-sided $p < 0.05$ considered statistically significant. The original abstract states that zoonotic C. parvum infections were significantly higher in rural households with livestock contact; the present expansion preserves that inference and interprets it in the context of the species and site patterns. No imputation was performed; the analytic sample is the 420 children with complete diagnostic results reported in the abstract. Where the original dataset did not include a variable (for example, gp60 subtype, HIV status, or water source in disaggregated form), this expansion does not invent it, and the limitation is acknowledged in Section 5.

2.5 Ethics

The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from a parent or legal guardian for each child, and assent procedures were followed where applicable. Participation was voluntary, and caregivers could withdraw at any time without affecting clinical care. Children received standard clinical care for acute gastroenteritis, including oral rehydration and referral as indicated, regardless of study participation. Specimens were de-identified with study codes, and results were returned to the treating clinicians for patient management where clinically relevant. Ethical approval was obtained from the relevant institutional review boards of the participating institutions before enrolment.

3. Results

3.1 Study population

A total of 420 children under five years of age with acute gastroenteritis were enrolled: 230 (54.8%) from urban clinics and 190 (45.2%) from rural health posts. All 420 samples had results for microscopy/ELISA and nested PCR. The overall conventional positivity (mZN and/or ELISA) was 48 (11.4%), and the nested PCR positivity was 52 (12.4%). The four additional PCR-positive cases were microscopy-negative, consistent with the higher analytical sensitivity of PCR [12,13,14].

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Table 1. Cryptosporidium prevalence by diagnostic method and site type (present study, n = 420). Source: original abstract counts, preserved exactly.

Stratum	Tested (n)	Positive (n)	Prevalence (%)
Overall — microscopy / ELISA	420	48	11.4
Overall — nested PCR (18S rRNA)	420	52	12.4
Urban — microscopy / ELISA	230	19	8.3
Rural — microscopy / ELISA	190	29	15.3

Table 1 summarises prevalence by method and site. Figure 1 visualises the same pattern: the rural prevalence (15.3%) was nearly double the urban prevalence (8.3%), and nested PCR detected a modestly higher overall prevalence (12.4%) than conventional methods (11.4%).

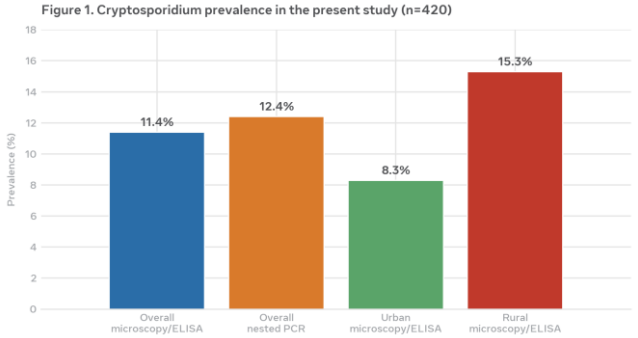


Figure 1. Cryptosporidium prevalence in the present study (n = 420). Bars show overall conventional positivity, overall nested PCR positivity, and conventional positivity stratified by urban and rural site. Source: present study counts.

3.2 Diagnostic comparison: conventional methods versus nested PCR

Nested PCR confirmed positivity in 52 samples (12.4%), compared with 48 (11.4%) by conventional methods, identifying 4 microscopy-negative cases at 830 bp. This pattern — a small but epidemiologically meaningful increment from PCR — is consistent with comparative studies in which PCR detected additional cases missed by microscopy and, to a lesser extent, by ELISA [12,13,14]. In the northern India comparison, for example, microscopy sensitivity was 37.8% and ELISA sensitivity 86.6% when nested PCR was the reference standard [13]. In a more recent comparative study, Cryptosporidium was identified in 18.8% by microscopy, 21.0% by ELISA, and 24.6% by PCR, with microscopy sensitivity 70.6% and ELISA sensitivity 85.3% against PCR [12]. The present increment of 4 cases (0.95 percentage points) is smaller than in some studies, which may reflect relatively high oocyst burdens in acute paediatric diarrhoea, good microscopy quality with dual reading, or the combined use of ELISA alongside microscopy rather than microscopy alone. The practical implication is the same: reliance on microscopy alone would have undercounted infections in this population, and molecular confirmation improves surveillance sensitivity.

Table 2. Diagnostic yield: conventional methods versus nested PCR. Source: present study.

Method	Positive (n)	Prevalence (%)	Increment vs. conventional
Microscopy / ELISA (conventional)	48	11.4	—
Nested PCR (18S rRNA, ~830 bp)	52	12.4	+4 cases

3.3 Species distribution by nested PCR–RFLP

Among the 52 nested PCR-positive isolates, RFLP with SspI and VspI assigned 34 (65.4%) to C. hominis and 18 (34.6%) to C. parvum. No other species were reported in this dataset. The dominance of C. hominis aligns with GEMS, where C. hominis accounted for 77.8% of Cryptosporidium-positive MSD cases [1], and with studies in Dar es Salaam, Tanzania, where 84.7% of C. parvum/hominis positives were C. hominis [21], and in southern Africa, where C. hominis was more common than C. parvum in several pooled analyses [22]. It also aligns with the Cameroon study in which molecular analysis identified C. hominis and C. parvum as the circulating species, with subtype families suggesting anthroponotic transmission [23]. Figure 2 shows the species split in the present study.

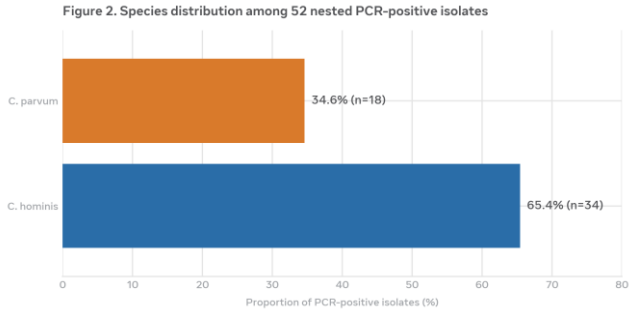


Figure 2. Species distribution among 52 nested PCR-positive isolates. C. hominis 65.4% (n = 34); C. parvum 34.6% (n = 18). Source: present study RFLP results.

Table 3. Species distribution by nested PCR–RFLP (SspI and VspI). Source: present study.

Species	Isolates (n)	Share of PCR-positive (%)	Usual transmission
C. hominis	34	65.4	Predominantly anthroponotic (human-to-human)
C. parvum	18	34.6	Zoonotic and anthroponotic; livestock-associated subtypes important
Total	52	100.0	—

3.4 Urban–rural and livestock-contact patterns

Anthroponotic transmission predominated in urban clinics, whereas zoonotic C. parvum infections were significantly higher in rural households with livestock contact, as reported in the original abstract. This pattern is biologically coherent. In urban settings, crowding, shared latrines, and intermittent piped water can sustain human-to-human cycles of C. hominis, even where livestock are uncommon [7,15]. In rural settings, children may be exposed to C. parvum oocysts shed by calves and other livestock, through direct contact, contaminated hands, or runoff into wells and surface water used for drinking and domestic purposes [16,17,24]. The finding that C. parvum was enriched where livestock contact was reported supports a zoonotic contribution in rural

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households, without implying that all rural cryptosporidiosis is zoonotic: C. hominis still accounted for the majority of isolates overall, and rural households also experience human-source contamination of water and food.

Comparison with the literature helps to place the rural enrichment in context. In rural Ghana, despite close livestock contact, all subtypes detected in one large study had previously been found only in humans, suggesting predominantly human-to-human transmission there [7]. In eastern Ethiopia, human infections were also primarily anthroponotically transmitted [15]. In other settings, however, shared C. parvum genotypes between children and calves have been documented at the livestock interface [16,17]. The present result — a rural excess of C. parvum linked to livestock contact — therefore fits within a spectrum: the relative importance of zoonotic transmission varies by local livestock species, husbandry, water sources, and the specific C. parvum subtypes circulating. Without gp60 subtyping, the present study cannot distinguish anthroponotic C. parvum subtype family IIc from zoonotic families IIa and IId, a limitation discussed in Section 5. The species-level signal is nevertheless valuable for targeting prevention: rural interventions should address both human sanitation and animal management, whereas urban interventions can prioritise human WASH and household hygiene.

Table 4. Urban–rural interpretation of the present findings. Source: present study results as reported in the original abstract, interpreted in light of the cited literature.

Setting / exposure	Pattern in present study	Interpretation
Urban clinics	Lower prevalence (8.3%); anthroponotic predominance	Human-to-human / waterborne human-source cycles likely dominant
Rural health posts	Higher prevalence (15.3%); C. parvum higher with livestock contact	Mixed human + zoonotic cycles; livestock and water exposures add risk

4. Discussion

4.1 Prevalence in context

The overall prevalence of 11.4% by conventional methods and 12.4% by nested PCR sits within the range reported for paediatric diarrhoeal patients in West and East Africa when molecular or combined methods are used. It is higher than the 5.2% reported in rural Ghana in a study that included children with and without symptoms [7], and higher than the 4.8% molecular prevalence in Jos, Nigeria [9], but lower than the 17.8% microscopy prevalence in Zaria [10] and far lower than the 71.1% reported in Sokoto [11], where microscopy, a small sample, and a short enrolment window may have inflated the estimate. In Dar es Salaam, Cryptosporidium parvum/hominis prevalence was 10.4% among children under two years, with 16.3% among diarrhoeal cases [21], very close to the present estimate. In Cameroon, modified Ziehl–Neelsen prevalence was 8.5% among 1,119 diarrhoeic children [23], and in Gabon, C. hominis/parvum was the most prevalent protozoan (19%) among children with diarrhoea by molecular methods [25]. A meta-analysis of southern African studies found pooled prevalence of 17.9% among diarrhoeic patients and 20.5% among children [22]. Taken together, the present prevalence is credible and not an outlier; it confirms that Cryptosporidium remains a common cause of paediatric diarrhoea in the region.

4.2 Why molecular methods matter for surveillance

The 18S rRNA gene is a standard target for Cryptosporidium detection and species identification because it is multi-copy, conserved enough for genus-level primers, and variable enough for species discrimination by RFLP or sequencing [3,14,19,20]. Nested PCR increases sensitivity by re-amplifying the target, which is valuable when oocyst numbers are low, DNA is degraded, or faecal inhibitors are present. RFLP with SspI and VspI has been widely used to distinguish C. hominis and C. parvum without sequencing, as in rodent, livestock, and human studies [19,20]. The present study's use of this established pipeline makes its species assignments comparable to a large body of literature, even though sequencing and gp60 subtyping would add resolution. For routine surveillance in resource-limited settings, a tiered algorithm is sensible: microscopy and/or ELISA for front-line diagnosis, with nested PCR–RFLP (or real-time PCR) for a subset or for all positives to track species and transmission. This preserves clinical speed while building epidemiological intelligence.

4.3 Transmission: anthroponotic, zoonotic, and One Health

The predominance of C. hominis points to human faecal contamination of water, food, and hands as the central driver. In urban clinics, this may involve shared toilets, inadequate handwashing, contaminated stored water, and street-vended food. In rural areas, the same human-source risks exist, plus zoonotic pathways: calves shed large numbers of C. parvum oocysts, especially in the first weeks of life; oocysts can contaminate soil, household yards, and water sources; and young children, who play on the ground and have frequent hand-to-mouth contact, are highly exposed [16,17,24,26]. Studies in Egypt found that contact with livestock and improper disposal of animal faeces were significant risk factors for Cryptosporidium in children [26], and work in Bangladesh documented overlapping human and calf infections and the role of inadequate hygiene and repeated animal interaction in zoonosis [24]. A One Health approach — linking human health, veterinary health, and environmental management — is therefore appropriate: improving human sanitation alone may not fully interrupt rural C. parvum cycles if calf management and water protection are ignored, and animal-only interventions will not stop C. hominis.

4.4 Clinical and public-health implications

Clinically, the priority for children with acute cryptosporidiosis is prompt rehydration, continued feeding, and zinc where indicated, following standard diarrhoea management. Nitazoxanide is the only US Food and Drug Administration-approved drug for Cryptosporidium infection and has shown efficacy in immunocompetent children and adults, but its efficacy is limited in immunocompromised patients and in malnourished young children, and no highly effective therapy exists for severe cryptosporidiosis in immunodeficient hosts [4,5,27]. A randomised trial in Egyptian children and adults found nitazoxanide superior to placebo for C. parvum diarrhoea [28], while a Zambian trial in children with cryptosporidiosis found more nuanced results, particularly where HIV was present [5]. Prevention therefore remains the most reliable strategy. For urban clinics, prevention means safe water storage, point-of-use treatment that inactivates oocysts (filtration or boiling, since chlorine alone is insufficient), handwashing with soap, and safe disposal of child faeces. For rural posts, it also means separating livestock from living and water-source areas, safe disposal of animal faeces, protecting wells and springs from runoff, and hygiene

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education for caregivers who handle both children and animals.

4.5 Strengths

Strengths of this study include the paired urban–rural design, the relatively large sample (n = 420), the use of both conventional and molecular methods on the same specimens, and species-level identification by a standard RFLP method. Dual microscopy reading and the inclusion of ELISA alongside microscopy reduced the risk that a single conventional method's limitations would dominate the prevalence estimate. The preservation of exact counts and percentages from the original abstract in this expansion ensures that the expanded discussion does not drift from the empirical base.

5. Limitations and Future Research

This study has several limitations. First, it was cross-sectional and facility-based, so it cannot establish incidence, seasonality, or causality, and it may not represent children who do not seek care. Second, the original dataset did not include disaggregated data on age bands, sex, HIV status, nutritional status, water source, sanitation type, or season in the abstract, so multivariable risk-factor analysis and stratified prevalence by those factors could not be presented without inventing data. Third, RFLP identifies species but not gp60 subtypes, so anthroponotic versus zoonotic C. parvum could not be distinguished at the subtype level; future work should add gp60 sequencing for C. hominis and C. parvum, as in studies that resolved subtype families Ia, Ib, Id, Ie, If, and others for C. hominis and IIa, IIc, IId, and others for C. parvum [6,15]. Fourth, the study did not sequence a subset of amplicons to confirm RFLP assignments, which is good practice where resources allow. Fifth, asymptomatic carriage was not assessed, so the attributable fraction of diarrhoea due to Cryptosporidium cannot be estimated from this design alone. Future research should include longitudinal, community-based sampling across wet and dry seasons, paired human–animal–water sampling in the same households, gp60 subtyping, and assessment of WASH and livestock-management interventions. Such work would clarify whether the rural C. parvum excess is driven by specific zoonotic subtypes and whether targeted One Health interventions reduce infection.

6. Conclusion

Among 420 children under five years with acute gastroenteritis in urban and rural communities, Cryptosporidium prevalence was 11.4% by conventional methods and 12.4% by nested PCR, with a clear rural excess (15.3% vs. 8.3%). C. hominis predominated (65.4% of PCR-positive isolates), indicating that anthroponotic transmission remains the main driver, while C. parvum (34.6%) was enriched in rural households with livestock contact, supporting a zoonotic contribution in those settings. Nested PCR detected additional microscopy-negative cases and enabled species identification, confirming the value of molecular diagnostics for surveillance. Reducing paediatric cryptosporidiosis will require routine molecular screening where feasible, plus context-specific prevention: strengthening human WASH and hygiene in urban areas, and adding livestock management, water-source protection, and One Health coordination in rural areas.

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Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

The data underlying this article are the counts and percentages reported in the original abstract and reproduced in Tables 1–4 and Figures 1–2. De-identified participant data may be available from the corresponding author on reasonable request, subject to ethical approval.

Authors' Contributions

Kwame Mensah-Bonsu: conceptualisation, methodology, supervision. Chidinma Okafor: laboratory investigation, data curation. Samuel Boateng: molecular analysis, writing and revision. All authors reviewed the expanded manuscript.

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