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Analyzing the Mimics: A Comprehensive Examination of Cryptosporidium parvum Differential Diagnosis

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ABSTRACT

Cryptosporidium parvum is a pathogen that can lead to severe gastrointestinal disease, especially in neonates or individuals with weakened immune systems. Cryptosporidium parvum necessitates a minimal quantity of the pathogen to initiate infection and excretes a significant amount of it. The goal of this systematic review is to look at the many tests used to tell the difference between Cryptosporidium parvum and other diseases.

Method Choosing a database requires a multidisciplinary approach that makes use of resources like PubMed, Google Scholar, Cochrane Library, Embase, and Web of Science. The collection and processing of data entail the evaluation of feces samples and the comparative analysis of diagnostic performance, sensitivity, and specificity through statistical methodologies. It involved human studies with individuals suffering from diarrheal diseases and compromised immune systems, employing diverse diagnostic techniques including PCR. Samples were obtained from various geographic areas to guarantee a comprehensive representation of Cryptosporidium infections. We used PCR, ELISA, and microscopy to figure out the differential diagnosis. Data was gathered based on things like the size of the sample, the diagnostic methods used, the results, and the identification of Cryptosporidium parvum subtypes or species

Results: Results revealed that C. parvum can be diagnosed either microscopically or by immunochemistry techniques. While using molecular methods are usually being used for detecting specific strains of C. parvum and not for differential diagnosis.

Conclusion The transmission of Cryptosporidium parvum, a particular strain of Cryptosporidium, into the gastrointestinal tract and the subsequent development of cryptosporidiosis has been the subject of extensive scientific research.

KEYWORDS: Cryptosporidium parvum, differential diagnosis, Microscopy, ELISA, PCR.

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INTRODUCTION

Cryptosporidium parvum is one of the causative agents of intestinal disease that can be lethal for neonates and immunodeficient individuals (Xiao and Griffiths., 2020). *C. parvum* has a very low infectious dose and high shedding rates, and it is highly stable in the environment, which complicates the development of strategies to avoid its spreading. A lot of people use oproto-parasitological tests, such as Ziehl-Neelsen staining (ZN) and immunoenzymatic tests (ELISA test), to see if they have C. parvum infection (Lee et al., 2021) Moreover, PCR end-point diagnosis methods have also been used for a long time. In addition to this, electropherotyping methods that depend on PCR-sequence-based results have been tested for a few years (Lin et al., 2021), but a new promising technique that detects Cryptosporidium-antigen in stool samples is the enzyme-linked fluorescent assay (ELFA) system, which detects parasites independently of PCR centrifugation and/or immunostaining (Zhang et al., 2020). In this systematic review, we provided an analysis of the potential of different diagnostic alternatives other than the gold standard for *C. parvum* differential diagnosis.

Cryptosporidium parvum diseases, which usually display the high mortality in immunocompromised people and newborns, require prompt diagnosis and basic knowledge of other causes of diarrhea for a good prognosis. (Crawford & Kol, 2021). At this

point, this review will provide us with an analysis of the diagnostic potential of hyper-endemic and viral gastroenteritis (GE) in a clinical differential diagnosis setting (Bhilegaonkar & Kolhe, 2023). It should be noted that basic diagnosis relies primarily on the detection of liquids or photographic images of fecal parasites that have been stained with dyes (Nyenke et al., 2022). The low value of this methodology is often abandoned, and as a result, differential diagnosis is no longer possible in the clinical setting with other diarrheal diseases. (Schiepatti et al., 2020)

Cryptosporidium parvum, a species within the genus Cryptosporidium, is a pathogenic protozoan that can, although loosely, be classified in the Apicomplexa phylum right alongside other well-known disease agents such as Plasmodium species and Toxoplasma gondii due to its similar life cycle. (O'Leary et al., 2021).

In human feces, a single coccidial oocyst of *C. parvum* will on average contain 4 sporozoites, each carrying 4 infective sporozoites. (McDougald et al., 2020) The entire sporulated oocyst will measure 4–6 μm across with a roughly spherical shape. *C. parvum* develops through a complex life cycle that leads to the formation of gametocytes and the mixing of haploid genomes within the oocysts, which leave the host in its feces completing the cycle through water, soil, person-to-person, or zoonotic transfer. (Géba et al., 2021)

Cryptosporidium parvum differential diagnosis alone can present a significant challenge for diagnostic healthcare professionals, regardless of access to standardized diagnostic methods and materials given the massive overlap of its clinical presentation with multiple other potential disease makers. (O'Leary et al., 2021). Diagnoses often favor more common infections such as, in this paper, G. lamblia infections in clinical presentations that mirror *C. parvum* replication. (Chulanetra and Chaicumpa, 2021) The microbiology report on the patient can be completely accurate and show no clinical terrors resulting, but the original suspected culprit may have to now be isolated from samples for unnecessary periods of time (calamity and off-label drug prescriptions compounded by epidemiological alerts) while a physician eliminates all of the differential causes (Pulappadi et al., 2021).

This systematic review aims to investigate the different examinations used for differential diagnosis of *Cryptosporidium parvum*.

METHODOLOGY

Search Strategy

Developing a clear and effective research method is important to performing a thorough analysis of the differential diagnosis of *Cryptosporidium parvum*. This strategy involves coming up with the right search terms and synonyms, putting together full search strings, choosing the right databases, running searches, and carefully reviewing and writing down the search process.

Key Concepts

The initial step is to identify the principal concepts for the investigation. Some of them are "mimics," referring to diseases or conditions with analogous symptoms; "differential diagnosis," the process of determining the underlying issue with *C. parvum*; and "*Cryptosporidium parvum*," a protozoan parasite that induces gastrointestinal illness. Subsequently, it is necessary to generate pertinent search phrases and their synonyms. "*Cryptosporidium*" and *"C. parvum*" may substitute for "*Cryptosporidium parvum.*" The term "differential diagnosis" will now encompass the terms "diagnosis," "diagnostic criteria," and "differential." The term "mimics" will be employed alongside phrases such as "similar diseases" and "conditions."

Creating Search Strings

The search terms will be amalgamated with appropriate keywords to produce search strings that enhance the thoroughness of the search. Essential search strings may comprise combinations such as "Cryptosporidium parvum" AND ("differential diagnosis" OR "misdiagnosis" OR "diagnostic criteria") and "Cryptosporidium" AND ("mimics" OR "similar diseases" OR "differential"). Extended search strings would integrate more synonyms and complex combinations to ensure exhaustive coverage of the topic.

Database Selection

For database selection, a multi-disciplinary approach will be adopted. PubMed will be used for comprehensive biomedical literature, Google Scholar for broad academic articles, Cochrane Library for systematic reviews and meta-analyses, Embase for biomedical and pharmacological data, and Web of Science for a multidisciplinary citation database from 2018 to 2024. We found 2804 after filtration there were 14 studies.

Search Execution and Review

These databases will all use the search strings. In order to satisfy requirements particular to each database, searches will be conducted and keywords updated as needed. Further refinement of the findings will be achieved by using filters for publication date, language, and kind. The relevancy of the search results will be assessed after it has been executed. Based on preliminary results, the search keywords and approach will be adjusted to improve the accuracy and relevancy of upcoming searches. In order to find further sources, manual searches of references listed in relevant papers will be carried out. Ultimately, it is essential to maintain comprehensive documentation of the search procedure. This entails monitoring the search term, the database utilized, the date of the search, the quantity of results, and any pertinent articles identified. Modifications to the search plan will also be documented.

Data Collection and Analysis

We concentrated on diagnostic precision and methodological variety, incorporating 14 papers that met our inclusion criteria. We selected the studies based on their efficacy in differential diagnosis and their utilization of several diagnostic methodologies,

Including PCR, microscopy, and serological assays. To enhance comprehension of Cryptosporidium parvum's mechanisms and improve diagnostic techniques, it is essential to gather and analyze data. Fecal samples were collected from a diverse population, including children and individuals with compromised immune systems, across various global locations. Subsequently, various diagnostic methods were employed to analyze these materials, including microscopy, Polymerase Chain Reaction (PCR), and Enzyme-Linked Immunosorbent Assay (ELISA). The main aim of the data analysis was to evaluate the overall diagnostic performance, sensitivity, and specificity of these approaches.

Inclusion Criteria

The inclusion criteria for the studies on Cryptosporidium parvum were established to guarantee the accuracy and dependability of the data. Human studies were included. The study encompassed toddlers experiencing diarrhea and individuals with compromised immune systems, such as those afflicted with HIV or cancer. The investigations necessitated the utilization of various diagnostic procedures, including a minimum of one diagnostic tool such as PCR, to detect Cryptosporidium. Samples were gathered from multiple geographic regions to guarantee diverse representation of Cryptosporidium infections.

Exclusion Criteria

Samples from asymptomatic people were omitted, as the emphasis was on symptomatic infections. Studies using animals were omitted. Studies lacking diagnostic procedures were omitted due to the enhanced sensitivity and specificity of these approaches relative to conventional methods. Furthermore, samples from places with minimal prevalence of Cryptosporidium were omitted to preserve the study's emphasis on regions with considerable public health implications.

Differential Diagnosis

Diagnosing *Cryptosporidium parvum* differently from other gastrointestinal pathogens that elicit comparable symptoms, like diarrhea, is part of the diagnostic process. The accurate detection of Cryptosporidium oocysts in fecal samples necessitates the application of several diagnostic techniques. Research evaluated various diagnostic techniques, such as PCR, ELISA, and microscopy, to determine how well they differentiated Cryptosporidium from other infections. For instance, PCR was found to have higher sensitivity and specificity compared to microscopy and ELISA, making it a preferred method for differential diagnosis. Accurate differential diagnosis is critical for effective treatment and management of cryptosporidiosis, especially in vulnerable populations like young children, and immunocompromised individuals.

Selection of Studies

The chosen studies for this study investigated multiple facets of the diagnosis and epidemiology of Cryptosporidium parvum across varied populations and settings. Research utilizing diagnostic techniques such as PCR, ELISA, and microscopy to identify Cryptosporidium parvum in fecal samples meets the inclusion requirements. Only studies that offered adequate data on the sensitivity, specificity, and accuracy of these diagnostic methods were approved. Studies that did not yield pertinent diagnostic results or were limited to non-Cryptosporidium parasites were excluded.

Extraction of Data

Data was retrieved from each selected study, emphasizing key factors such as sample size, applied diagnostic techniques (e.g., microscopy, ELISA, PCR), diagnostic outcomes (e.g., sensitivity, specificity), and the discovery of any Cryptosporidium parvum subtypes or species. The supplementary data encompassed the study's geographical setting, the age demographics of the participants, and pertinent findings related to the prevalence or epidemiological parameters linked to *Cryptosporidium parvum* infection.

Quality Evaluation

A variety of parameters, including study design (e.g., prospective versus retrospective), adequate sample size, methods for diagnostic validation, and data presentation, were employed to assess the quality of each study. Studies with larger sample sizes and those employing molecular techniques such as PCR were deemed superior due to their potential for enhanced sensitivity and specificity in detecting Cryptosporidium parvum. The evaluation of quality considered the accuracy and completeness of data reporting, as well as the statistical methods used to measure diagnostic accuracy.

Limitations

The chosen research indicated negligible limitations. This encompassed potential biases in sample selection (such as convenience sampling), alterations in study populations (including age demographics or geographical areas) that could influence generalizability, and variations in diagnostic methodologies along with their intrinsic sensitivities and specificities. Moreover, comparing the diagnostic efficacy of various approaches proved challenging due to the utilization of distinct "gold standards" for Cryptosporidium detection in each investigation. The interpretation of the results included the constraints of the retrospective design of certain studies and the potential for publication bias.

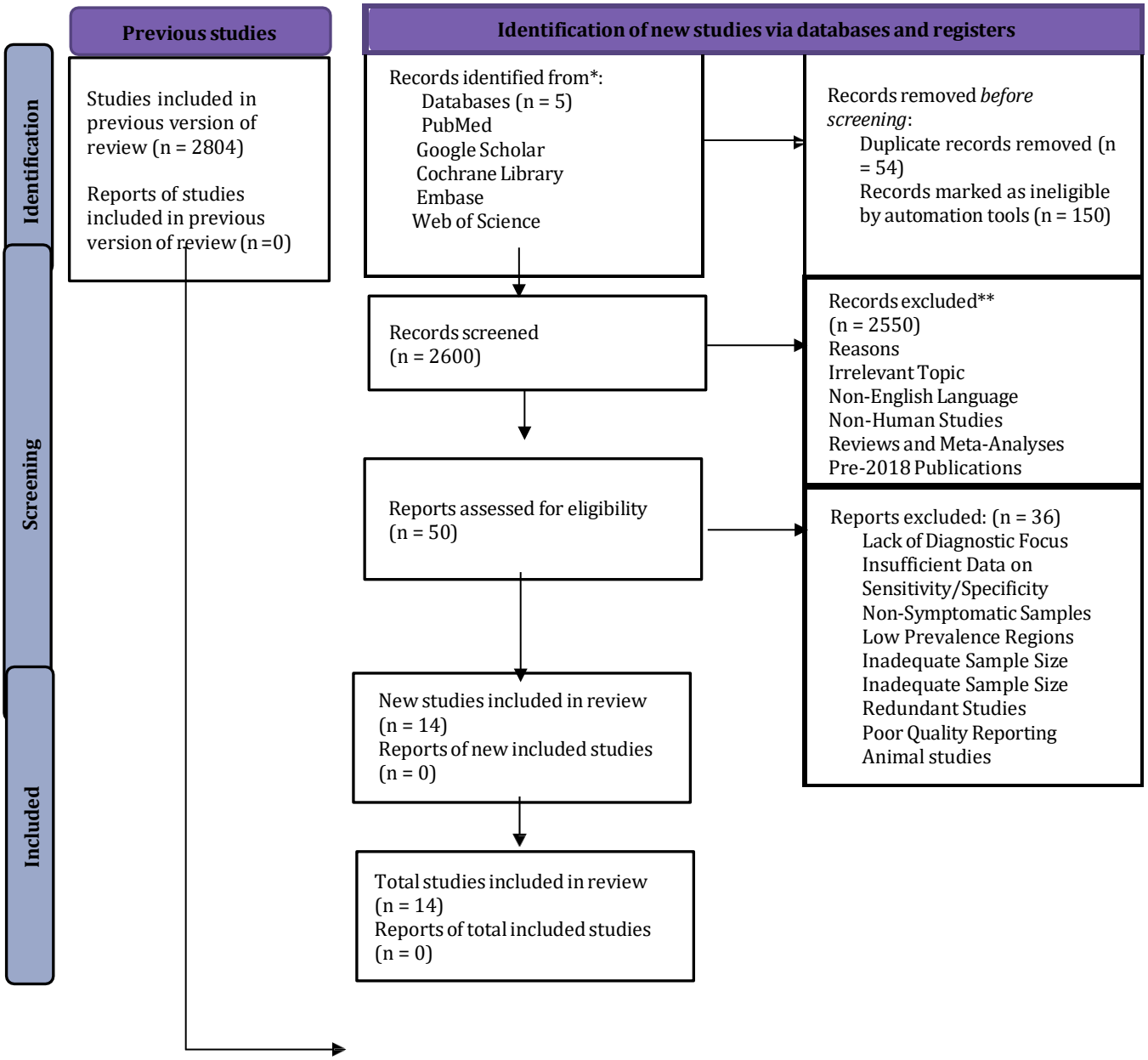

Figure (1) Prisma flow chart

RESULTS

Author	Date	Sample	Method	Key findings
Shin, J. H., et al	2018	Three types of waterborne protozoan parasites: Cryptosporidium parvum, Cyclospora cayetanensis and Giardia lamblia.	The TaqMan probe approach was used to detect three target genes in various parasite infection cases. These genes include Cryptosporidium oocyst wall protein for C. parvum, glutamate dehydrogenase for G. lamblia, and internal transcribed spacer 1 for C. cayetanensis. The gene product 21 from bacteriophage T4 was employed as an internal control DNA target to	The study devised a new primer-probe combination for each parasite, a mixture of primers and probes for both the parasites and the internal control, to be used in multiplex real-time PCR analysis. Additionally, a procedure for implementing this detection approach was established. The primer-probe cocktail used in multiplex real-time PCR

			assess the amplification of human feces DNA. TaqMan probes were synthesized with four distinct fluorescent dyes, namely FAM™, HEX™, Cy5™, and CAL Fluor Red® 610, for the detection of C. parvum, G. lamblia, C. cayetanensis, and bacteriophage T4, respectively.	effectively and accurately identified the target genes of C. parvum, G. lamblia, and C. cayetanensis in a mixed spiking human stool sample. The assay's limit of detection was 2×10 copies for C. parvum and C. cayetanensis, and 2×103 copies for G. lamblia. The multiplex real-time PCR detection method established in this study is a valuable approach for detecting the most prevalent protozoa responsible for traveler's diarrhea simultaneously.
Mahmoudi, M., et al.	2020	221 stool samples from patients had diarrhea	Two hundred twenty-one samples of diarrheal stool were collected from children admitted to Motahari Hospital in Urmia, Iran, for research purposes. Methods such as AF staining and ELISA were employed; nonetheless, PCR was the preferred technique. Sequencing was performed on positive samples to identify the species of Cryptosporidium. The three methodologies were evaluated according to statistical characteristics, sensitivity, specificity, predictive values, duration, and experimental expenses.	The AF staining and PCR methods indicated that four and seven samples, respectively, were positive for Cryptosporidium. The AF staining technique exhibited a sensitivity of 57.14% and a specificity of 99.53%. The ELISA approach accurately identified five of 94 samples as positive, with a sensitivity of 71.4% and a specificity of 100%.
Manouana, G. P., et al.	2020	596 stool sample	A cross-sectional study was performed at four locations in Sub-Saharan Africa (Gabon, Ghana, Madagascar, and Tanzania) between May 2017 and April 2018. Stool specimens were collected from children under the age of 5 who had either had diarrhea or had a recent history of diarrhea within the preceding 24 hours. The specimens underwent processing and analysis using CerTest Crypto RDT, which was compared to a composite diagnostic panel consisting of two	A study involving 596 stool samples from Ghana, Gabon, Madagascar, and Tanzania evaluated the effectiveness of the CerTest Crypto RDT for detecting Cryptosporidium species. The test's sensitivity varied significantly across the sites, with Madagascar having the highest at 72.22%. However, the test's specificity remained consistent across all locations. The study also examined the test's performance throughout the year, considering seasonal

			polymerase chain reaction (PCR) tests (qPCR and RFLP-PCR). The composite diagnostic panel served as the gold standard.	and batch variations. Despite inconsistent false negative cases, there was no discernible pattern suggesting a batch influence on the test performance. No evidence was found to suggest gender, rainfall, sample period, or age group had any effect on Cryptosporidium infection. However, a significant prevalence of Cryptosporidium infection was observed in children under two across many countries.
Shrivastava, A. K., et al.	2020	Commercially sourced C. parvum oocysts were obtained from Waterborne Inc (New Orleans, LA, USA). The DNA of C. hominis, C. andersoni, and C. felis parasites was acquired from the Christian Medical College, Vellore (India) upon request.	The development and validation of highly accurate and precise SYBR green quantitative PCR (qPCR) and TaqMan qPCR assays were carried out utilizing the newly found targets TU502HP-1 and TU502HP-2, at both the diagnostic and analytical levels.	The test validation results demonstrated that the newly developed real-time PCR assays exhibit 100% specificity and a dependable limit of detection. The assays demonstrated excellent repeatability and reproducibility, yielding high-quality data in both intra- and inter-laboratory analyses.
Messa Jr, A., et al	2021	190 confirmed positive samples of Cryptosporidium	The "Global Enteric Multicenter Study" (GEMS) in Mozambique identified a proportion of 11.5% (430 out of 3754) using the ELISA method. These samples were then successfully amplified and sequenced using PCR at the gp60 or ssu rRNA loci to determine the species and genotype.	Cryptosporidium species, such as C. hominis, C. parvum, and C. meleagridis, were detected in Mozambique, with a higher prevalence of infections observed in children aged ≤ 23 months. Both species had a higher occurrence rate among youngsters suffering from diarrheal illness. Significant genetic variation was found among C. hominis and C. parvum, but not C. meleagridis. No correlation between Cryptosporidium species/genotypes and the age of the child was found. The overwhelming prevalence of C. hominis and C. parvum IIc indicates that these illnesses are mostly transferred from humans to humans. Additional molecular epidemiology research is needed to examine the contribution of

				livestock, poultry, and other domestic animal species to environmental pollution and the occurrence of cryptosporidiosis in humans.
Pestechian, N., et al.	2022	187 samples collected from cancer patients	Oncology patients' feces were studied at Isfahan University of Medical Sciences. The study included 187 samples. A modified Ziehl-Neelsen acid-fast procedure was used to stain the samples before they were inspected under a microscope. In order to extract DNA from positive samples, they were preserved in 2.5% potassium dichromate. The glass beads and phenol chloroform technique were used to extract DNA. The 18S rRNA gene was used in a nested PCR to identify Cryptosporidium spp. Subtypes were subsequently determined for positive samples by employing nested PCR to amplify gp60 sequences. For the purpose of identifying Cryptosporidium spp. isolates, the derived nucleotide sequences were compared to reference sequences stored in GenBank.	4.3% of the participants (n=8) tested positive for Cryptosporidium spp. In a study including eight samples, five were confirmed to be Cryptosporidium spp. by nested PCR for the 18S rRNA gene. Two subtypes of C. parvum, IIaA18G3R1 (n = 2) and IIaA17G2R1 (n = 2), were found through sequencing of the gp60, while one subtype of C. hominis, IbA6G3, was uncovered. Very few other studies have found the IbA6G3 subtype.
Dumas Marucci, et al.	2024	30 patients with “Disease Associated with Immunosuppression” (DAI)	Children at the Hospital de Pediatría S.A.M.I.C. in Buenos Aires, Argentina, who were diagnosed with DAI and cryptosporidiosis, were the subjects of a case series report. Free tertiary care is available at this hospital, which specializes in neonatology, organ transplants, and cancer and blood disease treatment. The presence of Cryptosporidium oocysts in stool samples was used to define cryptosporidiosis. Researchers looked at cryptosporidiosis patients' electronic health records and found	From January 2018 to April 2023, a total of 30 patients were recorded with both diffuse axonal injury (DAI) and cryptosporidiosis. Among these patients, there were 19 males and 11 females. The median age of the patients was 7 years. Out of the 30 patients, 18 had persistent diarrhea and 7 had acute diarrhea. Six patients had co-infection with multiple microorganisms. Additionally, 13 patients had previously undergone solid organ transplants, 8 had hematologic neoplasms, and 4 had primary

			out things like their sex, age, underlying illness, treatment type, length of hospital stay, and frequency and severity of diarrhea episodes. Additionally, we looked at cases of co-infection with various parasites, viruses, or bacteria that cause diarrhea.	immunodeficiency. Two patients received hematopoietic stem cell transplantation (HSCT) for the treatment of chromosome disease, two had solid tumors, and only one patient with cryptosporidiosis had HIV infection. At the time of diagnosis of cryptosporidium infection, 25 out of the 30 patients were hospitalized, with a median length of stay of 17 days.
Mergen, K., et al.	2020	The study involved 92 stool samples collected over a 5-month period from February to March.	The study encompassed the analysis of 92 fecal samples that were gathered over a span of 5 months, specifically from February to March. The specimens were concentrated using Fecal Parasite Concentrator Kits and subsequently resuspended in a solution containing 10% formalin. Subsequently, the stool was utilized to cleanse slides and left to dry overnight in preparation for staining and microscopy. The samples underwent modified acid-fast staining and direct fluorescent antibody labeling using a Cryptosporidium/Giardia detection kit. In order to establish the detection thresholds for the GI panel and the LDT, a solution containing C. parvum was added to phosphate-buffered saline (PBS) and stool samples. The feces were conserved in the alcohol-based fixative Total-Fix and subsequently subjected to washing and extraction processes. The process of extracting DNA was carried out utilizing a conventional stool extraction methodology on the automated DNA extractor known as easyMAG.	A commercial gastrointestinal panel (GI panel), immunofluorescent microscopy, an antigen-based detection quick test, and modified acid-fast microscopy were all used to compare the molecular test's results. Only 20 of the 40 positive samples were detected by microscopy, while 21 were detected by antigen-based approaches. Although not all specimens were received in the required preservative, the GI panel nevertheless found 33 out of 40 positive samples. Out of forty samples that tested positive, the LDT identified Cryptosporidium. Our results show that the LDT is the most cost-effective, dependable, and accurate way to detect Cryptosporidium in a clinical public health reference laboratory.

			PCR amplification was conducted using a QuantaBio 5X ToughMix, and real-time reactions were carried out using a ViiA7 RT-PCR system. 10 µL of extracted DNA was tested in a final reaction volume of 25 µL, and all specimens were tested twice. An further set of replicated assays was conducted, using 1 µL of extracted DNA, in order to assess the presence of inhibition.	
Razakandrainibe, R., et al.	2021	50 oocysts positive samples	Researchers looked for Cryptosporidium spp. in three French medical parasitology labs: one in Rouen, one in Nantes, and one in Dijon. The French Cryptosporidium National Network supplied 50 stool samples with Cryptosporidium oocysts, and 12 and 28 fresh C. parvum oocyst-positive random samples were also used. After confirming the diagnosis under the microscope, the species of Cryptosporidium was identified by polymerase chain reaction (PCR) at the 18S ribosomal DNA locus. Five new stool samples were also included in the investigation, and the counts of C. parvum oocysts ranged from four to seventy-six per fifty microscopical fields. Following the manufacturer's instructions, the researchers conducted the ELISA in triplicate to reduce the possibility of handling errors and reading mistakes. Optical density (OD) measurements of 3,3′,5,5′-Tetramethylbenzidine (TMB) products at 450/605 nm were used to express the results. According to Loong, the study evaluated	The effects of chilling and K2CRO4 treatment on ELISA antigen detection in clinical samples were examined in preliminary investigations. The optical density (OD) values of undiluted stool, undiluted stool diluted in PBS, and undiluted stool stored at -80°C were all identical. Antigen positivity was confirmed in all samples. The number of C. parvum oocysts in feces was used to establish the ELISA detection cut-off value for clinical samples. The two labs found similar optical densities (OD) for fifty feces that tested positive for oocysts. Six out of seven negative controls tested positive for ELISA, and four out of six samples were determined to be negative under the microscope but positive when tested with PCR. Since the ELISA test for Strongyloides stercoralis ova came back negative, the possibility that Lab#2's false-positive sample tested positive for this parasite was rejected.

			specificities, sensitivity, positive predictive value, and negative predictive value.	
Akgün, S., & Çelik, T.	2020	Between January 2016 and August 2018, our hospital's laboratory received 362 stool samples from patients at various clinics who presented with gastrointestinal system problems. These samples were subsequently reviewed retrospectively.	Researchers looked for parasites in patient stool samples using the native-lugol technique. The identification of Cryptosporidium parvum, Giardia intestinalis	The following were detected in fecal samples using the EPP assay: Cryptosporidium hominis Morgan-Ryan, 1912; Fayer, Thompson, Olson, 2002; Lal et Xiao, 2002; and E. histolytica. Alternatively, kinyoun's acid-fast was used to stain samples that tested positive for Cryptosporidium spp. Of the 362 stool samples, 41 (11.3%) tested positive for parasites, while 23 (6.3.3%) tested positive using EPP assay. Fifteen samples tested positive for G. intestinalis, whereas twenty-two samples (6.1%) tested positive for E. histolytica and Entamoeba dispar. C. parvum or C. hominis was found in three samples, accounting for 0.8% of the total. When tested using both the EPP assay and Kinyoun's acid-fast technique, only two samples came back positive. While the EPP assay is easy to use, it is still no substitute for a microscope when diagnosing amoebiasis. Microscopy was shown to be just as accurate for the diagnosis of G. intestinalis and C. parvum/C. hominis.
Jassim, A. H., & Al-Mussawi, K. A.	2021	200 cases of diarrhea, intestinal colic, and stomach pain, 200 samples were taken	The procedures that were employed were chromatographic immunoassay, flotation with saturated sugar solution, and modified Zell Nelson staining. A wood stick is spread on a glass slide, fixed with methanol, then CarbolFoxin dye is added. A calm flame is let to sit for 20 minutes in the modified Zell	Among the methods of infection diagnosis, immunochromatographic analysis yielded the best detection rate (7.5%). The infection rate was 6% when the flottening method was used, and 4% when the microscopic inspection was done. Eight out of two hundred samples tested positive for Cryptosporidium parasite eggs; the

			Nelson staining. After the samples were rinsed with tap water and stained with blue malachite, they were examined at a 100x magnification. To prepare for floating in a saturated sugar solution, feces must first be filtered using distilled water and several layers of gauze. Centrifuge the filtrate at 100 rpm for 10 minutes afterward. Once the silt is well-mixed with the sugar solution, the supernatant is removed and added. The next step is to spin the filtrate at 1000 rpm for 10 minutes in a centrifuge. Each sample was assigned to one of three life stages: less than one year, one to three years, and more than three years. Staining can be detected visually by chromatographic immunoassay, often known as Cer test crypto. In a specific hole, 50 µl of the serum sample is tested, and then 50 µl of the dilution solution is added to the same hole. Anyone can see the difference in color.	prevalence was greatest in children ages one to three. The transmission of the parasite was greatly influenced by the age factor. An infection rate of 1 per 54 samples was recorded for children less than one year old, 9 per 105 for children aged one to three years, and 2 per 105 for children older than three years. Children had the greatest infection rate in the immunological scan, which was attributed to their poor nutrition and lack of personal hygiene. According to the results, the age factor is crucial for the parasite's dissemination.
Hijjawi, N., Zahedi, A., & Ryan, U.	2021	159 sample of patients who suffers from diarrhea	A study obtained 159 fecal specimens from individuals experiencing diarrhea and abdominal cramps in five different locations of Jordan between November 2014 and October 2016. Demographic data, including age, gender, residency, and medical history, were acquired. Genomic DNA was isolated from all samples and analyzed for the presence of Entamoeba spp., Cryptosporidium spp., gp60 loci, and Blastocystis spp. The DNA fragments that were amplified were	The study examined the occurrence of Entamoeba, Blastocystis, and Cryptosporidium species in 159 samples using Quantitative Parasitology 3.0 software. The overall incidence of Entamoeba spp. was 19.5%. Out of the fecal samples, 20 were found to have Entamoeba spp., with 17 isolates of E. dispar, two isolates of E. histolytica, and one case of mixed infection with both E. histolytica and E. dispar. The data was validated at the 18S rRNA site, with the exception of

			separated through the process of agarose gel electrophoresis and then purified for sequencing at each specific location using an ABI Prism™ Dye Terminator Cycle Sequencing kit. The Sanger sequencing chromatogram files were imported into Geneious Pro 8.1.6 software. The nucleotide sequences were then examined and matched with reference sequences from GenBank using the Clustal W algorithm.	one E. dispar sample that did not amplify at the actin site. Blastocystis hominis was found in 6% of the samples, but Cryptosporidium parvum was only isolated in one sample. There were no instances of mixed infections with the three parasites that were investigated. However, one sample showed coinfection with both E. histolytica and E. dispar.
Basmaciyan, L., et al.	2021	The Parasitology Laboratories of Dijon University Hospital submitted a total of 173 DNA samples.	The investigation entailed retrieving DNA samples from fecal matter following microscopic analysis and preserving them at a temperature of -20°C until the extraction process. Each sample was substantial enough to conduct 10 PCR experiments within a single defrost cycle lasting up to 48 hours, hence preventing any bias caused by DNA degradation. The LightCycler 2.0 thermocycler was utilized for in-house SimpPCRa, while the performance of commercial kits was assessed using the same thermal cycler. The gold standard for data analysis was conducted by microscopical examination. The results obtained from the in-house SimpPCRa and the microscopical investigation were consistent, and no PCR inhibitors were found using the commercial kit DIAControlDNATM. The in-house SimpPCRa was deemed to be equally efficient as the microscopical inspection, making it a viable option to be used as the gold standard. The SimpPCRa, which was conducted internally, was used for data	The performance of four commercially available SimpPCRa kits (specifically, CerTest-VIASURETM) and three MultPCRa kits (specifically, CerTest-VIASURETM, FAST-TRACK-Diagnostics-FTD-Stool-ParasiteTM, and DIAGENODE-Gastroenteritis/Parasite-panel-ITM) was assessed for the detection of Cryptosporidium spp., Entamoeba spp., and Giardia intestinalis in stool samples, in comparison to our regularly used in-house SimpPCRa method. On a global scale, the SimpPCRa shown higher sensitivity and specificity in detecting G. intestinalis, E. histolytica, E. dispar, and Cryptosporidium spp. (96.9% sensitivity and 93.6% specificity for G. intestinalis, 100% sensitivity and specificity for E. histolytica, 95.5% sensitivity and 100% specificity for E. dispar, and 100% sensitivity and 99.3% specificity for Cryptosporidium spp.), compared to the three commercial MultPCRa that were tested. In summary, we have demonstrated that MultPCRa provides a

			analysis in order to compare each PCR assay with one another.	compelling alternative for detecting protozoans in stool samples, depending on the specific clinical situation.
Al-Sherefy, M. K., & Al-Hamairy, A. K.	2022	96 positive samples were tested by PCR	A cross-sectional study was conducted. A total of 987 stool samples were evaluated using the direct smear method, and 96 positive samples from these were further analyzed using the polymerase chain reaction technique (PCR). This study included individuals of all genders and age groups (children and adults) who were diagnosed with diarrhea and received treatment at Babylon Maternity and Children Hospital, Specialized Marjan Hospital for Internal and Cardiac Diseases in Babylon province, as well as basic health care facilities and private clinics. and for individuals ranging in age from under one year to 31 years and older	The present investigation revealed that the prevalence of parasitic infections causing diarrhea was 47.3%, with Entamoeba histolytica, Giardia lamblia, and Cryptosporidium sp. accounting for 26.4%, 17.9%, and 3.7% of the cases, respectively. The polymerase chain reaction (PCR) was employed to identify the pathogens responsible for causing diarrhea, specifically G. lamblia, E. histolytica, and Cryptosporidium sp., in samples that were positive under microscopic examination. The results showed an overall infection rate of 43.4%, with individual rates of 31.3% for G. lamblia, 28.1% for E. histolytica, and 2.2% for Cryptosporidium sp.

DISCUSSION

Accurate differential diagnosis of *Cryptosporidium parvum* is instrumental in terms of both practical patient management and public health concerns. This point has been underscored in the literature many times within the last decade (Hassan et al., 2021). However, the percentage of facilities that have adequate data with the pathogens is just around 10% for Cryptosporidium, suggesting the requirement for analogous data on probable differential diagnosis mimics. (Manouana et al., 2020) As it is, few articles mention potential differential diagnosis for *C. parvum*, and frequently these references only include a single report. (Wang et al., 2021)

Common differential diagnosis mimics include atypical *C. parvum*, but some common false positives may include the genetically and pathologically similar C. hominis, as well as E. bieneusi and Cystoisospora sp. (Muadica et al., 2020) Given the long-standing zoonotic and anthroponotic character of this pathogen, it will behoove clinical and public health efforts to conduct an accurate differential diagnosis of Cryptosporidium as frequently as resources will allow. (Ryan et al., 2021)

The potential to diagnose this pathogen can change clinical management resulting from improvements in supportive care for affected individuals. It is critical that current reports employ advanced technology that is not inhibited by inhibitors against additional PCR-free diagnosis because such data will not be helpful in examining the mimics. (Hassan et al.2021) also be collaborative to enhance the percent of PARASIGHT readings showing contaminants that appear as Cryptosporidium oocysts (Cystoisospora, Montereyensis) from typical non-biopsy stool specimens. (Mthethwa et al., 2022) This will provide an analogous level of suspicion for cryptosporidiosis as is currently facilitated for patients with bronchoalveolar lavage cytology consistent with the diagnosis of pneumocystosis for undercover surveillance of PCP. (Davidson et al., 2020)

Symptoms of *Cryptosporidium parvum*, as discussed by epidemiologists, include profuse, watery diarrhea of progressive severity that lasts longer than three days. (Khan & Witola, 2023). It may also be associated with anorexia, abdominal cramps, low-grade or absent fever, weakness, malaise, nausea, myalgia, vomiting, weight loss, fatigue, headaches, photophobia, arthralgia, and cough. (Kozyolkin et al., 2023). There may also be a waxing and waning of symptoms. The presence of central nervous system, pulmonary, biliary, and/or gastrointestinal symptoms is characteristic of *Cryptosporidium parvum*. (Pradhan & Karanth, 2023).

Clinical signs of *Cryptosporidium parvum*, as reported by practitioners, include mild to moderate dehydration and mild to severe loss of body tone. (Morelli et al., 2021) Other clinical signs can be noted, including an abnormal blood count and/or weight loss. Abnormal stool studies can reveal steatorrhea, malabsorption of fat, and occult blood. (Montoro-Huguet et al., 2021) Symptoms and signs of *Cryptosporidium parvum* tend to overlap with symptoms and signs typically reserved for giardiasis or other parasitic, bacterial, and viral infections. (Khurana et al., 2021). Therefore, sole examination of a patient's stool for final diagnosis is not only insufficient for ruling in/excluding cryptosporidium but can lead to a misdiagnosis since many healthcare practitioners are apt to conclude that the pathogen is Giardia lamblia. (Downes et al., 2021).

Examination of patient stool for Cryptosporidium oocysts is the confirmatory diagnosis for cryptosporidiosis, and typically oocysts (unsporulated and oval) measure 4 to 6 micrometers in diameter (Pinto et al., 2022). Of the two genera, Eimeria is the most common coccidia considered in the differential intestinal diagnosis in animals, presenting with characteristic sporocysts in their stool. (Abd El-Ghany, 2020; Mesa-Pineda et al., 2021)

Microscopy has long been recognized as the gold standard for the diagnosis of cryptosporidiosis. However, like other morphologically indistinguishable species, such as C. wrairi, this approach has many disadvantages (Aboelsoued & Abdel Megeed, 2022). Stool may or may not contain oocysts that are excreted intermittently or in varying amounts. Even under the most acidic conditions, they may not be easily identified in simple zinc sulfate flotation methods. (Kimeli et al., 2020). The most often used dye contrast and optically dense Kinyoun's acetic acid techniques are three small µm oocysts slightly acid-resistant and cannot be identified with other pathogens or certain treatments with a layer of wax and easy oocyst and debris destruction. (Akgün & Çelik, 2020)

More recently, other stains and media have been developed to detect Cryptosporidium spp. and giardia specimens, such as Combo-Quick, Crypto-Giardia-Quick, modified WOW-CARB software, etc. Programs, as well as ELISA, do allow for easy processing of the laboratories and facilitate concurrent analysis of other diagnoses (Fradette et al., 2022). However, immunodiagnostic tests give negative reactions against morphologically typed parvum samples by PCR. The detection time of oocysts in stool must be considered in diagnosis. Failure to detect cryptosporidiosis in a sample of stools taken during the stool excretion period requires a second or third sampling of diarrhea (Khurana, S., Gur, R., & Gupta, N., 2021). But samples must be taken within four consecutive days because the quantity of intermittent crapshots in animals and people is high (Vanathy, K., et al., 2017).

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The microscopic examination performed on stool samples has been considered "the gold standard" for detection and, consequently, the identification of Cryptosporidium oocysts. For light microscopy observation, it is common to use a variety of staining methods, different for commercial availability, ease management, good level of detection of cryptosporidia, and acceptable contamination of stained slides with the endogenous fecal material of natural origin (Abdou, N. M. I., et al., 2022). Microscopic observation is performed at the morphological level of cell shape, cell content density, presence/absence of refractile body, and features concerning oocyst pedicels, micropyle, and oocyst wall. Additionally, the evaluation of oocyst measures

rounded to each single value among the various data ranges available in the literature is requested, together with an assessment of structural variation margins possible in the measurement of oocyst characteristics, data considered of interest in the differential diagnosis of cryptosporidia (Razakandrainibe, R., et al., 2021).

Immunodiagnostic techniques play an important role in the laboratory differential diagnosis of *Cryptosporidium parvum* infection (Khurana, S., & Chaudhary, P., 2018). Immunodiagnostics are nonconclusive methods used occasionally in research areas, but they are not applicable for practical application in differential diagnosis because of their low sensitivity and/or specificity and the cross-reactivity and false-positive results with infections by other protozoa and even by Cryptosporidium species (Abdou, N. M. I., et al., et al., 2022).

PCR is becoming increasingly popular for diagnosing cryptosporidiosis, replacing microscopy due to its laborious and tedious nature. The detection and research of Cryptosporidium have been previously hindered by the parasite's intractable nature. However, recent advancements have started to modernize research in this field (O'Leary, J. K., Sleator, R. D., & Lucey, B., 2021).

Currently, routine studies on cryptosporidiosis do not include identifying the species of the isolates. The focus is on confirming which Cryptosporidium species is important in the spread of this illness among humans. Molecular methods also allow for determining the potential for Cryptosporidium spp. to be transmitted from animals to humans, providing crucial information for understanding transmission in both endemic and epidemic areas. The most common molecular technique used for detecting and classifying Cryptosporidium is PCR, which amplifies DNA from purified oocysts (Ryan et al., 2021).

CONCLUSION AND FUTURE DIRECTIONS

Cryptosporidium parvum, a specific strain of Cryptosporidium, has been the focus of intense scientific investigation regarding its transmission into the gastrointestinal tract and the resulting condition known as cryptosporidiosis. It is distinguished by its coarse external layer and the release of oocysts through external shedding, which is exclusively seen in *C. parvum*. The ingestion of *C. parvum* contaminated with feces can lead to the development of a distinct coccidian parasite in animals. Since this idea was initially proposed in 1985, there has been a significant increase in the amount of research on this topic in relation to humans.

Future study should prioritize investigating the off-label use of in-drop medicines for the purpose of differentiating *C. parvum*. Additional areas of interest encompass the growing capacity of *C. parvum* to transmit diseases between animals and humans, the existing criteria for identifying cryptosporidiosis at livestock facilities, and the possibility of diverse clinical manifestations in cases of cryptosporidiosis. Conducting targeted study in these areas is crucial to evaluate the logical acceptability of the current *C. parvum* diagnostic paradigm, without necessarily exacerbating the issue of the untested standard.

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