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Cryptosporidium spp. in Immunocompromised Patients Attending the Infectious Diseases Clinic: Genetic Characterization and Public Health Risk Assessment

Enfeksiyon Hastalıkları Kliniğine Başvuran Bağışıklığı Baskılanmış Hastalarda *Cryptosporidium* spp.: Genetik Karakterizasyon ve Halk Sağlığı Risk Değerlendirmesi

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ABSTRACT

Cryptosporidium spp. remain a significant parasitic concern, particularly among patients with compromised immune function. Among those most affected are patients living with HIV/AIDS, recipients of organ transplants, and individuals undergoing chemotherapy for cancer. These groups are not only more susceptible to infection but also often experience prolonged and severe disease courses. Although transmission primarily occurs via the fecal-oral route, the ubiquity of Cryptosporidium in various environments, along with its zoonotic potential, presents significant challenges for effective containment. Recent literature has revealed a striking degree of genetic diversity within Cryptosporidium species, complicating efforts to trace infection sources and understand host-specific dynamics. Despite advancements in molecular diagnostics, their integration into routine clinical and public health workflows remains incomplete. This review brings together research published between 2019 and 2025, with an emphasis on genetic characterization techniques, laboratory biosafety concerns, and broader implications for infection prevention strategies. By outlining key developments and identifying areas where knowledge remains limited, this work aims to inform more targeted and effective interventions for safeguarding immunocompromised populations.

Keywords: *Cryptosporidium* spp., immunocompromised hosts, genetic diversity, biosafety, public health, molecular diagnostics

ÖZ

Cryptosporidium spp., özellikle bağışıklık sistemi baskılanmış hastalar arasında önemli bir paraziter sorun olmaya devam etmektedir. HIV/AIDS ile yaşayan hastalar, organ nakli alıcıları ve kanser tedavisi gören (kemoterapi uygulanan) bireyler bu durumdan en çok etkilenen gruplar arasında yer almaktadır. Bu gruplar yalnızca enfeksiyona daha duyarlı olmakla kalmaz, aynı zamanda enfeksiyonu daha uzun ve şiddetli seyirlerle yaşama eğilimindedir. Bulaşma esas olarak fekal-oral yolla gerçekleşse de, Cryptosporidium'un çeşitli ortamlardaki yaygınlığı ve zoonotik potansiyeli, etkili kontrol önlemlerinin uygulanmasını güçleştirmektedir. Son literatür, Cryptosporidium türleri arasında dikkat çekici düzeyde genetik çeşitlilik olduğunu ortaya koymuş ve bu durum enfeksiyon kaynaklarının izlenmesini ve konakçıya özgü dinamiklerin anlaşılmasını zorlaştırmıştır. Moleküler tanı alanında önemli gelişmeler kaydedilmiş olsa da, bu tekniklerin rutin klinik ve halk sağlığı uygulamalarına entegrasyonu henüz tamamlanmamıştır. Bu derleme, 2019 ile 2025 yılları arasında yayımlanmış araştırmaları bir araya getirmekte olup; genetik karakterizasyon teknikleri, laboratuvar biyogüvenliği ile ilgili hususlar ve enfeksiyon önleme stratejilerine dair geniş kapsamlı etkiler üzerinde durmaktadır. Önemli gelişmeleri özetleyerek ve bilgi eksikliği bulunan alanları belirleyerek, bağışıklığı baskılanmış bireyleri korumaya yönelik daha hedefli ve etkili müdahalelere katkı sağlamayı amaçlamaktadır.

Anahtar Kelimeler: *Cryptosporidium* spp., bağışıklığı baskılanmış konakçılar, genetik çeşitlilik, biyogüvenlik, halk sağlığı, moleküler tanı yöntemler

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1. INTRODUCTION

1.1. Background and Significance of *Cryptosporidium*

Cryptosporidiosis, caused by protozoa of the genus *Cryptosporidium*, has emerged as a major concern for global public health, particularly in the care of immunocompromised patients. Individuals with impaired immune defenses, including those affected by HIV/AIDS, patients receiving chemotherapy, or recipients of organ and stem cell transplants, often face heightened susceptibility to this infection. Among such patients, the disease frequently manifests as persistent and severe diarrhea, contributing to nutritional deterioration and a diminished quality of life (Colford et al., 1996; Mann and Okhuysen, 2023).

The primary modes of transmission include consumption of contaminated food and water, or direct contact with infected sources, both human and animal. In areas with limited access to clean water and adequate sanitation, the risk of infection becomes significantly elevated (Ahmadpour et al., 2020). Furthermore, asymptomatic carriers can facilitate unnoticed outbreaks in healthcare settings such as oncology and transplant wards, making infection control a complex task (Ahmed et al., 2022).

What makes *Cryptosporidium* particularly challenging is the considerable genetic diversity observed among its species and genotypes. This diversity not only influences host range and pathogenic potential but also affects the reliability of conventional diagnostic tools (Liu et al., 2015). In response, researchers have increasingly turned to molecular techniques like PCR and gene sequencing, which offer more sensitive and specific ways to detect and classify infections. These approaches allow for better understanding of transmission dynamics and support targeted interventions.

The present review is rooted in the necessity to address gaps in both clinical management and public health preparedness. It seeks to consolidate existing knowledge while advocating for broader integration of genetic characterization methods in surveillance, diagnosis, and biosafety assessments to reduce the burden of disease in vulnerable patient populations.

Recent research trends in *Cryptosporidium* have reflected this shift—from clinical observations to genomic surveillance and immunological investigations—highlighting the expanding scope and depth of inquiry in the field (Figure 1).

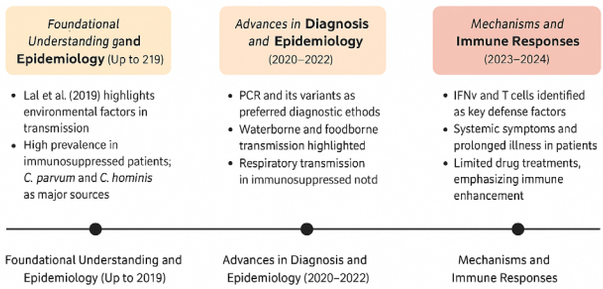

Figure 1. *The timeline of major thematic focuses in Cryptosporidium research (2019–2024) shows the shift from early epidemiological studies and microscopic diagnostics to the adoption of molecular tools, genotyping, transcriptomics, and One Health surveillance strategies (original).*

1.2. Objectives and Scope

This literature review sets out to explore how *Cryptosporidium* infections affect immunocompromised individuals, with particular focus on the ways in which genetics, diagnostics, and public health risks intersect in this context. The objective is not only to summarize existing findings but also to draw attention to trends and gaps that could shape future research and clinical strategies.

The primary scope of this review centers on three pillars: the use of molecular tools for genetic characterization, the assessment of biological risks associated with *Cryptosporidium* handling in clinical and research laboratories, and the practical application of findings in real-world healthcare settings. Much of the reviewed literature discusses how PCR and DNA sequencing have advanced our ability to identify species-specific infections and understand transmission routes, particularly the distinction between zoonotic and anthroponotic pathways (Audebert et al., 2020).

Given the resilience of *Cryptosporidium* oocysts in the environment and their high infectivity, biosafety emerges as a recurring theme in the studies analyzed. Clinical laboratories and healthcare institutions must navigate the risks of occupational exposure, especially when dealing with high-risk patient populations (Innes et al., 2020).

To ground these themes in practice, the review includes case studies from transplant units and other high-risk environments where proactive screening, patient education, and rapid diagnostics have shown promising results. These examples highlight how applied research can lead to tangible improvements in patient outcomes (Ahmed et al., 2021; Mann et al., 2023).

2. METHODOLOGY

In order to establish a sound academic foundation for this review, the literature was collected through a carefully structured and targeted search process. The objective was to identify relevant studies that investigate *Cryptosporidium* spp. infections in immunocompromised individuals, with a particular focus on molecular identification, genetic variation, diagnostic technologies, and biosafety implications. Rather than relying on a single source, a wide range of databases was utilized to capture both international and national publications, ensuring that the literature reviewed reflected the diversity of global and regional scientific contributions.

Internationally recognized databases such as PubMed, Scopus, Web of Science, and Google Scholar were used to locate English-language peer-reviewed articles in the fields of infectious diseases, clinical microbiology, and molecular diagnostics. At the same time, national academic sources, including TR Dizin and ULAKBİM, were actively searched to identify Turkish-language research, particularly from local medical faculties and public health institutions. A small number of additional studies in Chinese were retrieved

through CNKI to ensure that relevant data from China were not overlooked.

This review covered studies published between early 2019 and March 2025. This range was chosen deliberately to prioritize recent findings and methodologies, especially as the field has undergone considerable advancements in genetic analysis and laboratory biosafety standards over the past few years. The terms used to search the databases were adapted to both English and Turkish, and typical combinations included words such as *Cryptosporidium*, genotype, molecular diagnosis, PCR, and sequencing, alongside contextual terms like HIV/AIDS, transplantation, chemotherapy, and biosafety. Logical operators such as AND and OR were applied where appropriate, and efforts were made to adjust wording according to the indexing rules of each database.

Once the initial list of publications was obtained, abstracts and titles were reviewed manually to determine relevance. Those clearly aligned with the scope of this hypothesis were selected for full-text analysis. Literature was stored and organized using EndNote 20, which provided structured referencing and eliminated duplicate entries. Zotero was also used as a supplementary tool to group studies by thematic focus, particularly when comparing diagnostic techniques or genotypic diversity across patient groups.

The inclusion of a study in this review was not based merely on its topical relevance, but also on the quality and clarity of its design and presentation. Priority was given to articles written in English or Turkish that provided complete methodological details and were published in journals recognized by SCI, SCI-E, or indexed nationally in TR Dizin or ULAKBİM. In evaluating quality, factors such as citation frequency and the academic standing of the publishing journal were taken into account. Studies with clearly explained molecular methods, such as PCR primer sets or DNA sequencing workflows, and those that incorporated biosafety protocols in their design, were especially valued. Conversely, materials that lacked methodological transparency, relied exclusively on animal models without human application, or were not peer-reviewed were excluded from consideration. The same applies to overly dated publications or those dealing only with immunocompetent individuals without comparative relevance.

This methodological approach reflects both the breadth, and the precision required for a meaningful literature review. By integrating data from multiple languages and sources, and applying a consistent yet flexible standard of academic scrutiny, the review aims to construct a reliable and representative knowledge base for understanding *Cryptosporidium* infections in vulnerable patient populations.

3. REVIEW OF KEY THEORIES AND RESEARCH FINDINGS

3.1. Core Theoretical Framework

Cryptosporidium spp. are intracellular protozoan parasites of the phylum Apicomplexa. Among over 40 known species, *Cryptosporidium hominis* and *Cryptosporidium parvum* are the primary causes of human cryptosporidiosis, accounting for more than 90% of clinical cases globally (Ryan, Feng, and Fayer, 2021). These parasites complete their life cycle within a single host, undergoing both asexual and sexual reproduction inside the intestinal epithelial cells (Walzer et al., 2024).

After ingestion of infective oocysts—typically through contaminated water or food—sporozoites are released in the small intestine, where they attach to the apical surface of intestinal epithelial cells. Rather than fully invading the cytoplasm, the parasite occupies a unique intracellular but extracytoplasmic compartment called the parasitophorous vacuole. This location allows the parasite to evade host immune defenses while maintaining access to host-derived nutrients (Balendran et al., 2024).

In immunocompetent individuals, infections are usually self-limiting due to a coordinated immune response involving both innate and adaptive immunity. However, in immunocompromised individuals, such as those with HIV/AIDS, cancer, or organ transplantation, this balance is disrupted. Such patients often experience long-lasting infections that may progress to systemic complications, including respiratory and biliary manifestations (Hunter and Nichols, 2002; Bouzid et al., 2013; Balendran et al., 2024). Immune evasion strategies employed by *Cryptosporidium* include modulation of host signaling pathways and suppression of interferon-mediated responses. For example, studies have shown that the parasite can interfere with IFN-γ signaling, a critical component in controlling intracellular pathogens, thereby promoting its survival (Walzer et al., 2024).

Advances in molecular diagnostics have significantly improved the identification and classification of *Cryptosporidium* species. The 18S rRNA gene remains the most commonly used molecular marker due to its combination of conserved and variable regions, while the gp60 gene is widely used for subtyping, particularly in epidemiological investigations (Ryan, 2021; Dąbrowska, 2023).

Such molecular techniques enable differentiation between human-to-human *(C. hominis)* and zoonotic *(C. parvum)* transmission routes. Their clinical utility is particularly evident in immunocompromised populations, where rare genotypes such as *Cryptosporidium meleagridis* or *Cryptosporidium felis* are more frequently encountered (Ryan, 2021; Dąbrowska, 2023).

The ability of the host to control Cryptosporidium infection relies heavily on a competent immune response, particularly at the mucosal level. Following ingestion, Cryptosporidium oocysts excyst in the small intestine and invade epithelial cells,

where they reside within an intracellular but extracytoplasmic vacuole. This process initiates an immune cascade, wherein CD4⁺ T cells play a central role through the secretion of interferon-gamma (IFN-γ). IFN-γ is essential for macrophage activation and for the containment and clearance of the parasite from the intestinal mucosa. In immunocompromised individuals, especially those with advanced HIV/AIDS or post-transplant immunosuppression, this pathway is severely impaired due to reduced CD4⁺ T cell counts and diminished cytokine responses. As a result, infection becomes chronic, often leading to prolonged diarrhea, malabsorption, and weight loss (Ludington and Ward, 2015; Pantenburg et al., 2008; Borad and Ward, 2010).

The key stages of this host–parasite interaction and the consequences of immune deficiency are summarized in Figure 2 (Figure 2).

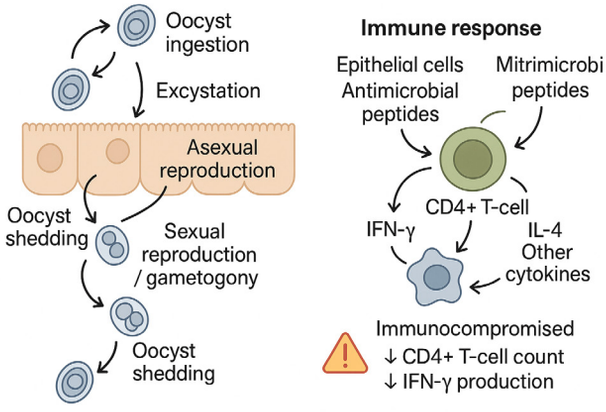

Figure 2. *Schematic representation of Cryptosporidium infection and host immune response. After ingestion, oocysts excyst and invade the intestinal epithelium, triggering immune responses primarily mediated by CD4⁺ T cells and interferon-gamma (IFN-γ). In immunocompromised individuals, this pathway is disrupted, leading to persistent infection (Original).*

3.2. Summary of Key Research Findings

Between 2019 and 2025, academic studies on *Cryptosporidium* infections in immunocompromised individuals have evolved in both depth and scope. Early investigations emphasized species identification and prevalence patterns, while recent work integrates molecular diagnostics, host-parasite interactions, and global prevention strategies.

Initially, attention was paid to the dominant species, particularly *C. parvum* and *C. hominis*, which are responsible for most human infections. These species were found to be more prevalent among immunosuppressed populations such as individuals with HIV/AIDS, transplant recipients, and patients undergoing chemotherapy (Balendran et al., 2024; Ryan, 2021). Differences in infection rates across countries were associated with sanitation standards and access to healthcare (Hunter and Nichols, 2002; Bouzid et al., 2013).

As molecular tools became more accessible, PCR-based diagnostics, including real-time PCR, significantly improved detection rates, especially for asymptomatic or low-load infections (Helmy and Hafez, 2022). Moreover, clinical descriptions expanded to include biliary and respiratory involvement, particularly in cases of advanced immunosuppression (Walzer et al., 2024).

The period from 2023 onward has seen increased focus on the genetic and immunological landscape of cryptosporidiosis. Transcriptomic and proteomic studies have uncovered distinct gene expression patterns between *Cryptosporidium* species and subtypes. These patterns may influence virulence and host specificity (Dąbrowska, Sroka, and Cencek, 2023).

Immunological research underscores the critical role of interferon-gamma (IFN-γ), IL-4, and T-cell-mediated responses in resolving infection. Individuals with compromised CD4+ T-cell levels typically experience more severe and persistent symptoms (Balendran et al., 2024; Bouzid et al., 2013).

Efforts to mitigate transmission have increasingly adopted the One Health model, integrating surveillance and intervention across human, animal, and environmental systems. This model is especially relevant in areas where zoonotic transmission contributes significantly to disease burden (Helmy and Hafez, 2022).

Ongoing research also prioritizes the development of new vaccines and low-cost diagnostic methods tailored to resource-limited settings. Genotype-specific risk assessments and treatment outcomes remain a key knowledge gap (Dąbrowska, Sroka, and Cencek, 2023; Ryan, Feng, and Fayer, 2021).

In conclusion, recent advances have expanded the understanding of cryptosporidiosis from a parasitological concern to a multidisciplinary public health issue, particularly in the context of immunocompromised care.

4. REVIEW OF RESEARCH METHODS AND TECHNIQUES

4.1. Commonly Used Research Methods in Medical and Research Laboratories

A wide range of diagnostic methods have been employed for the detection and characterization of *Cryptosporidium* spp., particularly in immunocompromised individuals who are more susceptible to severe outcomes. This section reviews the most commonly used classical and molecular techniques, evaluating their applicability, sensitivity, and limitations based on recent literature.

Microscopic techniques, particularly the Ziehl–Neelsen staining method, remain widely used as a first-line diagnostic tool in low-resource settings. Although its sensitivity is limited, this technique provides a cost-effective and rapid means of detecting Cryptosporidium oocysts in fecal samples (Helmy and Hafez, 2022). Its use is especially valuable for preliminary screening when molecular resources are not readily accessible.

Molecular diagnostic methods have significantly enhanced the detection of *Cryptosporidium*, especially in cases with low parasite burden, such as those seen in HIV/AIDS patients and other immunocompromised populations. Nested PCR targeting the 18S rRNA gene or other PCR methods commonly used in laboratories, such as real-time PCR, has been shown to offer high sensitivity and specificity in such contexts (Costa et al., 2021; Ryan, Feng, and Fayer, 2021). This technique enables the reliable detection of oocysts, even when present in very low concentrations.

Following PCR amplification, sequencing and genotyping further refine species-level identification. Genes such as 18S rRNA and gp60 are commonly targeted to distinguish among species like *C. parvum*, *C. hominis*, and others. These molecular markers are well-established tools in epidemiological surveillance and in elucidating transmission routes (Favennec et al., 2022; Ryan, Feng, and Fayer, 2021).

In addition to laboratory diagnostics, statistical tools have been employed in epidemiological studies to assess differences in infection prevalence across demographic groups. This statistical approach is particularly suitable for small to moderate sample sizes and has been extensively used in parasitological research (Hares et al., 2023).

Given the high environmental resistance and infectivity of *Cryptosporidium* oocysts, strict biosafety protocols are essential in laboratories conducting such work. Adherence to established biosafety guidelines is critical to ensuring the safe handling of potentially infectious specimens (Zhang et al., 2023).

Finally, comparative evaluations of various diagnostic techniques—taking into account factors such as cost, sensitivity, specificity, time requirements, and effectiveness in low-parasite-load or mixed infections—are crucial for selecting the most appropriate method. Table 1 summarizes key performance characteristics of commonly used detection and genotyping methods for *Cryptosporidium* spp. (Table 1).

Table 1. *Comparative analysis of molecular and microscopic methods used in Cryptosporidium detection and genotyping.*

Method	Sensitivity	Specificity	Cost	Processing Time	Detects Mixed Infections	Effective at Low DNA Load
Microscopy (Ziehl–Neelsen)	Low	Moderate	Low	Fast	No	No
PCR	High	High	Moderate	Moderate	Partial	Yes
qPCR	Very High	High	High	Fast	Yes	Yes
Nested PCR	Very High	High	Moderate	Slow	Yes	Yes

4.2. Case Studies and Comparative Analyses

The methodological choices in this research are supported by recent comparative studies and practical case applications.

For example, evaluation of eight PCR protocols found that nested PCR targeting 18S rRNA provided the best balance of sensitivity and robustness in detecting *Cryptosporidium* in clinical samples (Costa et al., 2021).

The superior resolution of next-generation sequencing (NGS), particularly in detecting and characterizing mixed infections, is acknowledged. However, its application in routine diagnostics is often constrained by higher costs and more complex technical requirements. In contrast, the PCR–sequence approach used here remains cost-effective and accurate for clinical and epidemiological purposes (Favennec et al., 2022).

Relying solely on microscopic examination may result in missed detection of low-intensity *Cryptosporidium* infections, particularly in immunocompromised individuals. This underscores the importance of integrating traditional staining techniques with molecular methods such as polymerase chain reaction (PCR) for accurate diagnosis. The combined approach is especially critical in vulnerable populations, including individuals living with HIV/AIDS, organ transplant recipients, and patients undergoing immunosuppressive therapy, where early and precise detection is essential for timely intervention. (Helmy and Hafez, 2022).

CRISPR-based screening studies have demonstrated that specific host cell genes are critical for the invasion and replication of Cryptosporidium, highlighting the importance of molecular-level approaches in elucidating the complex interactions between the parasite and its host. These findings not only enhance our understanding of host-pathogen dynamics but also offer potential targets for the development of novel therapeutic interventions. (Zhang et al., 2023).

Overall, the methodologies employed—ranging from microscopy to molecular diagnostics and statistical analysis—are consistent with current best practices in Cryptosporidium research. These approaches provide a scientifically robust framework for the detection and characterization of infections, particularly in immunocompromised individuals, where accurate and timely diagnosis is of critical importance.

5. CURRENT STATUS, CHALLENGES, AND FUTURE DIRECTIONS

5.1. Current Research Status and Evaluation

Over the past decade, research on *Cryptosporidium* spp. in immunocompromised individuals has increasingly focused on molecular detection methods, genotyping, and risk assessment in clinical and environmental settings. Current literature emphasizes whole genome sequencing (WGS), multilocus genotyping, and transcriptomics as key tools in identifying infection routes, strain diversity, and host-pathogen interactions (Dąbrowska, 2023).

Recent studies highlight a trend towards integrating genomic data into epidemiological surveillance. However, despite methodological advances, challenges remain regarding low oocyst concentrations in clinical samples and incomplete subtype detection (Imre et al., 2023). Regional studies, such as those conducted in Türkiye, Romania, and France, show that prevalence among HIV/AIDS patients can range from 6.3% to over 30%, indicating considerable regional

and methodological variability (Dărăbus et al., 2023; Elgun et al., 2022). Table 2, survey on *Cryptosporidium* prevalence among HIV/AIDS patients varies significantly across global regions, reflecting differences in diagnostics, environment, socioeconomic conditions, and host immunity (Table 2).

Table 2. *Average prevalence of Cryptosporidium spp. in HIV/AIDS patients across high-risk countries and Türkiye (Wang, 2018).*

Country/Region	Prevalence (%)
Cambodia	45
Haiti	41.6
Polan	28.6
South Africa	25.7
Mali	25.6
Czech	24.6
Cameron	22.9
Mexico	21.8
Cuba	21.5
France	30.0
Romania	6.4–16.7
Türkiye	2.6

At the same time, environmental studies are increasingly linked with public health assessments. *Cryptosporidium* presence in water sources and the influence of climate-related factors, such as rainfall and seasonal temperature variation, have been associated with increased outbreak risk (Boughattas et al., 2023). Novel approaches such as the use of zinc oxide nanoparticles in water disinfection systems have shown promise in reducing oocyst viability, although these technologies remain in the experimental phase (Elshamy et al., 2023). The study's experimental design, as depicted in Figure 3, outlines the complete sequence from sample acquisition to genotyping. There is a growing trend in literature toward incorporating genomic data into public health surveillance systems. Nevertheless, methodological challenges persist, particularly related to the detection of low oocyst burdens and mixed infections (Figure 3).

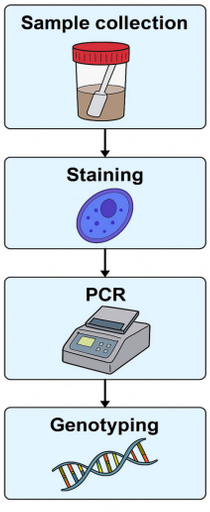

Figure 3. *Workflow of sample collection and laboratory processing for Cryptosporidium detection. Steps include fecal sample collection, microscopy screening, DNA extraction, nested PCR amplification, sequencing, and genotyping (original).*

5.2. Challenges and Problems

Despite technological developments, the field still faces considerable operational, diagnostic, and biological challenges. One primary issue is the difficulty of sustaining long-term in vitro culture systems, which limits access to high-quality DNA and hinders in-depth functional genomic studies (Imre et al., 2023; Chalmers, 2013). In addition, the existence of mixed infections, especially among immunocompromised individuals, often goes undetected by standard PCR assays, resulting in misclassification of subtypes (Cama, 2006; Gerace, 2019).

The lack of standardized genetic markers across research groups also poses difficulties in comparing global findings. Although the gp60 gene is widely used for subtyping, its variability limits its utility in broader evolutionary and transmission analyses (Baptista, Cooper, and Kissinger, 2021).

From a clinical perspective, access to advanced diagnostics remains limited in low-resource settings, where cryptosporidiosis is often underdiagnosed or misdiagnosed as general diarrhea, especially in patients receiving chemotherapy or antiretroviral therapy (Bouzid et al., 2018). Furthermore, risk assessments rarely incorporate environmental surveillance, despite well-documented associations between oocyst presence in surface water and disease incidence (Boughattas et al., 2023).

Laboratory biosafety concerns are also rarely addressed in research protocols. Studies have noted inadequate protective procedures when handling live oocysts, especially during high-throughput DNA extraction and amplification workflows (Elshamy et al., 2023).

6. FUTURE RESEARCH DIRECTIONS AND TRENDS

To address existing gaps, future research should prioritize the development of cost-effective, highly sensitive molecular diagnostics that are adaptable to both clinical and field settings. The integration of hybrid sequencing platforms, such as Oxford Nanopore and PacBio, will likely enhance the resolution of genotyping studies and support genome-wide association investigations (Dąbrowska, Sroka, and Cencek, 2023).

Research is also moving toward the adoption of One Health models, emphasizing the interconnectedness of human, animal, and environmental sources of infection. Improved environmental monitoring, paired with remote sensing and machine learning models, could enable real-time outbreak prediction and water quality surveillance (Boughattas et al., 2023).

Lastly, vaccine development remains an underexplored area, yet one of increasing interest, especially for pediatric and immunocompromised populations. Antigens expressed during the sporozoite and merozoite stages, as well as surface adhesion molecules, are currently being investigated for their immunogenic potential (Aboelsoued, 2022; Da Silva, 2021).

Building upon the molecular and epidemiological advances discussed above, the following section synthesizes key findings and outlines future research directions.

7. CONCLUSION AND OUTLOOK

7.1. Key Conclusions

This literature review examined *Cryptosporidium* spp. infections in immunocompromised patients through the lens of molecular diagnostics, genotypic surveillance, and public health risk assessment. Over the past decade, the molecular understanding of *Cryptosporidium* spp. has undergone substantial advancement, especially regarding its impact on immunocompromised individuals. This literature review highlights how genetic characterization techniques—particularly PCR, sequencing, and genome-wide studies—have greatly enriched our understanding of *Cryptosporidium* spp. diversity, transmission dynamics, and host specificity.

One of the most significant conclusions is that molecular epidemiology has enabled the precise identification of species and subtypes such as *C. parvum*, *C. hominis*, and *C. meleagridis*, with subtype IIaA15G2R1 being especially dominant in zoonotic cases (Pane and Putignani, 2022). These insights have not only informed clinical diagnostics but also helped delineate anthroponotic and zoonotic transmission pathways across diverse geographic regions.

Advanced tools like single-oocyst sequencing and long-read genome technologies (e.g., PacBio, ONT) now allow high-resolution analysis even in samples with low oocyst concentration, offering critical breakthroughs for both surveillance and treatment strategies (Widmer et al., 2020; Dąbrowska, Sroka, and Cencek, 2023). Transcriptomic and proteomic studies have further revealed the parasite's interaction with host pathways, including immune evasion and nutrient acquisition mechanisms, which are essential for understanding persistent infections in immunosuppressed patients (Jumani et al., 2021).

Moreover, *genetic profiling has proven essential in distinguishing* between clinical manifestations and epidemiological patterns across different risk groups. It provides valuable input for global health organizations in estimating disease burden and prioritizing interventions. In low-resource settings, genetic data can help validate cost-effective diagnostic strategies and improve risk modeling. Table 3 provides a structured comparison of multiple molecular studies on genotypic diversity and detection methods of *Cryptosporidium* spp. in immunocompromised populations (Table 3).

Table 3. *Comparative summary of recent molecular studies reporting genotypic profiles, diagnostic approaches, and detection rates of Cryptosporidium spp. in immunocompromised populations.*

Study	Population	Main Genotypes	Diagnostic Methods	Detection Rate (%)
Dărăbus et al., 2023	HIV patients (Romania)	C.hominis, C. parvum	Microscopy + PCR	6.4–16.7
Elgun et al., 2022	HIV patients (Türkiye)	C.parvum	Nested PCR	6.3–33.5
Imre et al., 2023	Cancer patients	Mixed (C. meleagridis)	Microscopy + ELISA + PCR	10–15

7.2. Recommendations for Future Research

Despite technological progress, significant gaps persist in *Cryptosporidium* research. One priority is the development of efficient long-term in vitro cultivation models, which remain a bottleneck in functional gene validation and drug screening (Jumani et al., 2021). Improvements in parasite culture systems are necessary to support high-throughput assays for vaccine candidates or therapeutic agents.

Additionally, the integration of multi-omics platforms genomics, transcriptomics, proteomics, and metabolomics should be pursued to explore complex host–parasite interactions and uncover new druggable targets. In particular, long non-coding RNAs and alternative splicing patterns represent promising but underexplored regulatory layers in parasite biology (Dąbrowska, Sroka, and Cencek, 2023; Li, Y et al., 2021).

Another critical area is the need for global standardized and scalable molecular surveillance frameworks. While countries like Japan and the UK have established subtype mapping networks, many regions with high cryptosporidiosis burden still lack reliable data. Comparative genomic studies show that C. hominis and C. parvum populations differ between countries, necessitating localized population genomic tracking to guide interventions (Widmer et al., 2020).

To strengthen public health response further, real-time outbreak detection using high-throughput genotyping and spatial-temporal data integration should be incorporated into surveillance systems. These approaches can aid early detection in healthcare and agricultural settings.

The proposed One Health-based framework visually presented in Figure 4 emphasizes an integrated strategy for tackling Cryptosporidium infections through four interconnected pillars: enhanced surveillance, research and innovation, policy development, and water and food safety. Enhanced surveillance calls for the implementation of molecular screening tools, particularly for immunocompromised individuals who are at higher risk. Simultaneously, advancements in research and innovation aim to improve diagnostic methods and deepen understanding of the parasite's pathogenesis. Insights gained from these efforts inform evidence-based policy development, where genotyping data can be utilized to guide public health strategies. Lastly, ensuring water and food safety remains critical to preventing environmental transmission. Collectively, these components converge under the One Health approach, highlighting the necessity of coordinated efforts across human, animal, and environmental health sectors. (Figure 4).

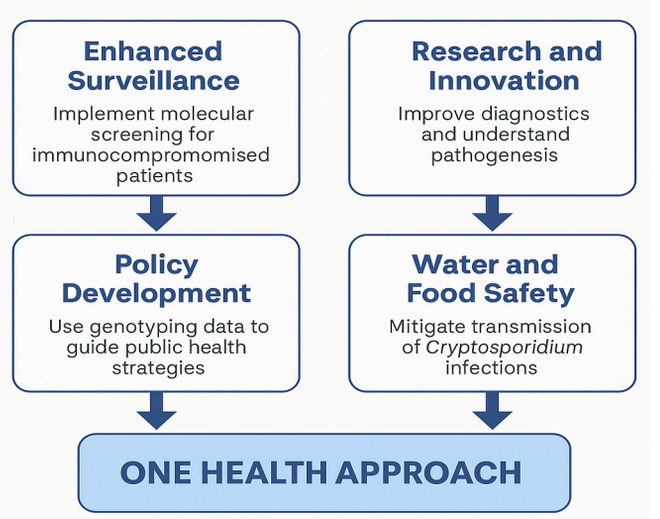

Figure 5. Proposed framework based on findings from this review

Figure 4. *One Health-based framework summarizing integrated prevention and control strategies for Cryptosporidium across clinical, veterinary, and environmental health sectors (original).*

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