Review

Cryptosporidium spp. Infection in Adult Kidney Transplant Patients

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Article

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Abstract: Diarrhea is a common occurrence following vascular organ transplantation, including kidney transplants. It can be induced by non-infectious factors, adverse effects of immunosuppressive drugs, or infectious agents such as viruses, bacteria, fungi, or parasites. Intestinal *protozoan parasites,* include *Cryptosporidium* spp., are *causes* of infectious *diarrhea* particularly in immunocompromised patients. This review aims to summarize reports on epidemiology, pathogenesis, clinical presentation, diagnosis, and treatment of *Cryptosporidium* spp. infection in adult patients, particularly following kidney transplantation.

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1. Introduction

In recent years, there has been significant improvement in the survival of kidney transplant recipients due to the prevention of acute rejection through immunosuppressive therapy. However, immunosuppressive treatment can lead to immune impairment, potentially resulting in opportunistic infections, including bacterial, viral, fungal, and parasitic infections. Protozoan pathogens, such as *Entamoeba histolytica*, *Giardia duodenalis*, *Cyclospora* spp., *Cystoisospora* spp., microsporidia such as *Enterocytozoon bieneusi*, and *Cryptosporidium* spp., are well-known causes of diarrhea and pose a major public health concern in developing countries [1]. *Cryptosporidium* spp. infection is identified as the second leading cause of diarrhea worldwide after rotavirus infection [2]. Deltombe et al. [3] found that the mean onset delay of *Cryptosporidium* spp. infection was 40.5 months post-transplantation.

Diarrhea is common after kidney transplantion, occurring in 10%-50% of patients [4]. It has been observed that diarrhea associated with immunosuppressive therapy is more prevalent in the early post-transplant period due to the administration of multiple drugs, often in higher doses. Conversely, infectious diarrhea tends to manifest several years after transplantation [5]. This review describes the epidemiology, pathogenesis, clinical presentation, diagnosis, and treatment of *Cryptosporidium* spp. infection in adult patients, particularly following kidney transplantation. The review is based on scientific articles sourced from validated databases such as PubMed and the National Centre for Biotechnology Information (NCBI), using keywords '*Cryptosporidium*' AND 'cryptosporidiosis' AND 'kidney' AND 'transplant'. Inclusion criteria encompassed human studies, in vitro animal studies,

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case reports, peer-reviewed journal publications, review articles, and research articles in English. Exclusion criteria included studies not in the English language, gray literature (e.g., conference proceedings and abstracts), and data related to pediatric patients.

2. Epidemiology of *Cryptosporidium* spp.

The coccidian protozoan *Cryptosporidium* spp. is an opportunistic intestinal parasite in vertebrate animals. *Cryptosporidium* infection has been documented in amphibians, reptiles, poultry, and mammals, including humans [6]. More than 40 *Cryptosporidium* species with over 60 valid genotypes have been identified [7]. *Cryptosporidium* spp. is recognized as an emerging protozoan parasite by the Centers for Disease Control and Prevention (CDC) [8]. Most human infections are caused by *Cryptosporidium parvum*, which infects both humans and ruminants, and *C. hominis*, infects humans and pigs [9]. Cases of cryptosporidiosis caused by other species and genotypes have also been reported. These include *C. meleagridis*, *C. felis*, *C. canis*, *C. cuniculus*, *C. ubiquitum*, *C. viatorum*, *C. muris*, *C. suis*, *C. fayeri*, *C. andersoni*, *C. bovis*, *C. scrofarum*, *C. tyzzeri*, *C. erinacei*, and *Cryptosporidium* horse, skunk, and chipmunk I genotypes [10]. Cryptosporidiosis has been documented in over 40 countries across six continents, affecting both immunocompetent and immunocompromised patients [11]. *Cryptosporidium* spp. infection is frequently observed in the *immunosuppressed patients*, including patients with HIV/AIDS, undergoing chemotherapy, or solid organ transplantation (SOT). Based on scientific data, the global prevalence of cryptosporidiosis is 7.6% [10]. The frequency of *Cryptosporidium* spp. infection depending on study design, area study, population group, sensitivity and and specificity of laboratory methods [12]. The prevalence of *Cryptosporidium* spp. in Europe and North America is 1%-3% and 5%-10% in Asia and Africa [13]. Several waterborne outbraks of cryptosporidiosis have been documented, with the largest reported in Milwaukee, Wisconsin (USA) in 1993, caused by *Cryptosporidium* oocysts that passed through the filtration system of one of two water treatment plants [14]. Over 400,000 people were affected by waterborne outbreak of cryptosporidiosis, including 100 immunocompromised patients.

3. Cryptosporidiosis

Cryptosporidium infection may be *asymptomatic* and self-limiting, which may contribute to its low detectability. In cases of asymptomatic infection, *Cryptosporidium* spp. has been found in the distal parts of the small intestine and proximal parts of the colon [15]. *Cryptosporidium* infection of the *gastrointestinal* tract *is most common,* respiratory cryptosporidiosis occurring much less frequently. The severity of the disease in immunosuppressed patients, including kidney transplant recipients, can be influenced by host factors such as age and nutritional status, as well as the species and subtype of *Cryptosporidium* spp. [16]. It has been observed that a $CD4^+$ T-cell count of <200/mm^3 increases the risk of prolonged infection, while counts of <100/mm^3 may lead to severe, life-threatening diarrhea [17]. Symptoms of cryptosporidiosis commonly appear 1-14 days (average 7 days) post infection (dpi) and persist up to 6-9 dpi [18]. However, some patients may experience symptoms for up to 100-120 days [19]. *Cryptosporidium* spp. infected patients excrete oocysts in feces for an average of 7 days (1-15 days) after discontinuation of symptoms, exceptionally, up to 2 months [20]. The *most common symptom* of *cryptosporidiosis* is *severe watery diarrhea* (up to 10 times a day), sometimes with mucus. Immunocompetent patients usually experience a self-limiting illness, while immunosuppressed patients, especially those with T-cell deficiency, often develop chronic and severe cryptosporidiosis with a risk of extra-intestinal disease development [21]. The pathophysiology of diarrhea in *Cryptosporidium* spp. infection remains unclear. It is suggested to be a combination of malabsorption and secretory diarrhea secondary to mucosal attachment, distortion of villous architecture, epicellular infection, inflammatory response, and cellular apoptosis [22]. In renal transplant patients, post-transplant cryptosporidiosis with diarrhea is a common complication [23]. Some researchers argue that in renal transplant recipients, cryptosporidiosis does not appear to be unusually severe or involve extraintestinal sites [24,25]. In the study by Deltombe et al. [3], cryptosporidiosis in renal transplant patients occurred at a mean time of 33.9 months, suggesting that patients were likely infected but asymptomatic prior to transplantation. Patients may also experience nausea, fever,

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vomiting, abdominal pain, anorexia, dehydration, weight loss (3.6±2.4 kg), and acute transplant dysfunction [3,26]. Immunocompromised patients may experience muscle pain, weakness, fatigue, malaise, headache, or anorexia [27]. *Respiratory tract infection* by *Cryptosporidium spp*. has been described for immunodeficient patients. The upper respiratory cryptosporidiosis may cause inflammation of the the nasal mucosa, sinuses, larynx, and trachea, accompanied by nasal discharge and voice changes, while lower respiratory cryptosporidiosis manifests as productive cough, dyspnea, fever, and hypoxemia [28]. Radiological changes visible in the lungs in the form of ground-glass opacities have also been observed. In most patients oocysts have been detected in both saliva and feces [29,30]. However, there is a lack of scientific literature on respiratory cryptosporidiosis in kidney transplant recipients. In immunodeficient patients *Cryptosporidium* infection may present *multiple organ* involvement. Generalized cryptosporidiosis has been described, among others, in an HIV-infected patient after kidney transplantation. The development of this form is facilitated by a high number of oocysts in the host's body [31]. In autopsy, material collected from immunodifiencet patient with generalized cryptosporidiosis caused by *C. baileyi* oocysts have been found in the esophagus, entire intestine, trachea, larynx, lungs, and gall and urinary bladders [32]. Recurrence of cryptosporidiosis symptoms in immunocompromised patients may occur even after treatment due to inadequate eradication (incorrect medication or dosage). This is caused by incomplete pathogen eradication, especially from the bile ducts, and maintenance of infection in the latent stage. Unrecognized cryptosporidiosis in immunocompromised patients can lead to debilitating diarrhea, epithelial infection of the bile ducts, gastritis, pancreatitis, primary sclerosing cholangitis, bile duct inflammation or cancer, and cirrhosis [12]. Cryptosporidiosis-associated biliary tract inflammation is a particularly serious clinical complication in AIDS patients. Symptoms include abdominal pain, fever, and jaundice. Patients exhibit increased alkaline phosphatase activity in serum and significant anatomical damage to the biliary system [15]. de Souza et al. [33] descrived a cryptosporidiosis of the biliary tract *clinically mimicking a pancreatic cancer in an AIDS patient.* In addition, *Cryptosporidium* spp. *can cause malignant cancers in the gastrointestinal* tract [34]. The type of immunosuppressants used in kidney transplant patients may influence the course of cryptosporidiosis (Table 1). It has been shown that patients using a tacrolimus-based regimen are at greater risk of *Cryptosporidium* spp. infection compared with a cyclosporine-based regimen [35]. In patients receiving tacrolimus with confirmed *Cryptosporidium* spp. infection, renal graft dysfunction is more common, possibly due to dehydration and increased tacrolimus concentration [36,37]. In patients receiving tacrolimus with diarrhea, blood levels of this drug should be assessed. Diarrhea, regardless of etiology, increases exposure to tacrolimus, which has a narrow therapeutic index. Elevated drug levels, combined with dehydration, can lead to graft dysfunction [38].

Cryptosporidiosis in Immunocompromised Patients

The National Reference Center in France between 2017 and 2019 reported that 40% of *cases notified* with *cryptosporidiosis* with documented immune status occurred in immunodeficient patients, including 53% in solid organ transplant recipients [39]. The majority of data regarding the prevalence of cryptosporidiosis in organ transplant recipients pertains to renal *transplant patients*. The prevalence of the *Cryptosporidium* spp. infections in adult kidney transplant recipients was 1.7%-53% (Table 1).

Table 1. *Cases* of *cryptosporidiosis* in a in adult kidney transplant patients based on scientific literature (TAC, tacrolimus; MMF, mycophenolate mofetil; PRED, prednisone; IL-2R, IL-2 receptor ; CNI, calcineurin inhibitor ; NTZ, nitazoxanide; AZM, azithromycin).

Patient age/gender	kidney transplantation		*Cryptosporidium* infection			Treatment of vryptosporidiosis		Reference
	Time after	Immunosuppressive treatment	Symptoms	Environmental risk factor	Species (intensity)	Drugs	Treatment result	
60	8 years /1st graft	TAC (4 mg/day) + MMF) (1 g x	watery diarrhea, nausea,	admitted to own a dog	*C. felis* (5–10 oocysts/slide)	NTZ (500 mg x 2/day for 14 days)	negative 2 weeks after treatment, with no recurrence of	[40]

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		2/day) + PRED(7.5 mg/day)	vomiting, weight loss (6 kg)				diarrhea observed 4 months	
64	2 years /1st graft	TAC (7 mg x 2/day), MMF (750 mg x 2/day), and PRED (10 mg/day)	watery diarrhea, abdominal pain, weight loss (13 Kg)	had travelled to Mali	*C. hominis* (>100 oocysts/slide)	1. reduction of TAC	diarrhea regressed after 8 days, 3 months after therapy stools still tested positive	
						2. NTZ (500 mg x 2/day for 14 days)	One month stools were tested negative	
34	8 years /2st graft	TAC (6 mg x 2/day), MMF (750 mg x 2/day), and PRED (25 mg/day).	watery diarrhea, abdominal pain weight loss (10 Kg)	had travelled to Kosovo	*C. parvum* (1–5 oocysts/slide)	1. reduction of TAC	diarrhea was regressing 1 month after treatment	
						2. NTZ (500 mg x 2/day for 14 days)	months after the second treatment course, his stools were tested negative	
38	2 years /1st graft	ND	diarrhea	ND	ND	Spiramycin 2 g daily for 10 days	Symptoms resolved second days therapy and reduction oocyst	[41]
42	1 year /1st graft	ND	Abdominal pain, distention	ND	ND	Spiramycin 2 g daily for 10 days	After treatment no symptoms and oocysts	
41/M	1 year /1st graft	ND	Weakness, fever of 39°C, yellowish diarrhea occurring 4-5 times daily without blood	ND	ND	NTZ 500 mg twice daily for 3 days.	successful recovery of *Cryptosporidium* spp. infection	[21]
37	1st graft	ND	acute diarrhea, up to 10 times daily, abdominal discomfort, coryzal symptoms	ND	ND	paromomycin 1 g twice daily + AZM 500 mg daily	1 week after treatment was still positive, after 4 weeks clinical and parasitological resolution	[42]
42/W	1 year	TAC (present levels 8–10 ng/ml), MMF (1250 mg/day) and steroids (PRED, 5 mg/day)	abdominal pain, diarrhoea (5 days)	travelled to Cuba	ND	rifaximin (600 mg thrice daily), reduced MMF to 1 g/day	patient's symptoms resolved within 1 week	[43]
24/M	1 month/ 2st graft	TAC + MMF	chronic watery diarrhea, nausea	ND	ND	NTZ monotherapy	ineffective	[44]
						NTZ (1 g twice daily) + AZM (600 mg daily) + rifaximin (550 mg twice daily) + intravenous fluids +	diarrhea within 10 days without recurrence	

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					diphenoxylate-atropine; AZM was stopped; TAC was 17 ng/mL, and it was discontinued and later restarted at a lower dose		
78/W	9 years /1st graft	Steroids 3 months post-transplant, TAC + MMF	watery diarrhoea without any pathological substance that had a 7 day evolution, without fever, vomiting or abdominal pain /sclerosing cholangitis			paromomycin + AZM of 14 days, subsequently NTZ for 6 days, doses of TAC and MMF were reduced	diarrhoea disappeared, kidney function recovered to basaline levels, 17 months later the patient was still asymptomatic	[45]
60	3 months	MMF/CsA/Cs, MMF switched to AZA	Severe diarrhoea			Spiramycin 10 days	Resolved	[46]
59	1 month	Sir/FK/Cs	Severe diarrhoea			Paromomycin 4 weeks	Resolved	[47]
68/M	56 months /1st graft	Anti-IL2r CNI + MMF	Diarrhea, vomiting, dehydration, weight loss (8 kg), acute kidney injury, acidosis	Contact with animals and children	C. parvum	Reduced MMF or stopped until diarrhea resolved, 500 mg of NTZ twice a day for four weeks	for the three patients gastrointestinal disorders resolved in the first two weeks; MMF was newly initiated as soon as diarrhea resolved; no relapse was observed	[3]
42/F	25 months /1st graft	Anti-IL2r CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (4 kg)	Previous antibiotic therapy with amoxicillin clavulanic acid	*Cryptosporidium* spp.			
77/M	14 days /1st graft	Anti-IL2r CNI + MMF	Severe diarrhea, dehydration, weight loss (3 kg), acute kidney injury	Contact with untreated water	*C. parvum*			

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53/M	2 days /1st graft	Anti-IL2r CNI + MMF	Diarrhea, vomiting, dehydration, weight los (4 kg)	Contact with cat	*C. felis*
64/F	65 months /1st graft	Depleting therapy CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (2 kg)	None	*C. parvum*
37/F	57 months /3st graft	Desensitization, depleting therapy CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (3 kg)	Work as a nurse, contact with recreative water, treated with phenoxymethylpenicillin	*C. parvum*

Cryptosporidium spp. infection is restricted to the epithelial cells of the small intestine, with the parasite primarily confined to the apical brush border. The infection, particularly in immunosuppressed patients, can also affect the pharynx, esophagus, stomach, appendix, colon and rectum, gallbladder and pancreatic ducts [45,48]. *Cryptosporidium* spp. have been reported in respiratory system mucosa, particularly patients infected by HIV or following bone marrow transplantation, as a complication of gastrointestinal infection [12,28,49,50]. The *monoxenous life cycle* of *Cryptosporidium* spp. consisting completing their entire life cycle within a single host. Both asexual and sexual stages develop in the intestinal epithelium, leading to the release of two forms of oocysts. Thin-walled oocysts are responsible for autoinfection, whereas thick-walled oocysts are released with the feces to hosts [51]. Oocysts containing sporozoites are excreted in the feces of hosts. The infectious dose was estimated to be 10-30 oocysts [52], although some researchers suggest that even a single oocyst carries *a* probability of *infection,* particularly in immunocompromised host [53]. *Cryptosporidium* oocysts *can* transmit through direct or indirect human-to-human or animal-to-human and through contaminated water and food. *Transmission* may *occur* by ingestion of animal contact, consumption of contaminated water or recreational water, travel to disease-endemic regions, poor hygiene, or foodborne transmission [53]. Airborne transmission and mechanical transport of *Cryptosporidium* spp. oocysts by flies and other insects have also been postulated [20,54,55]. The dissemination of *Cryptosporidium* spp. oocysts in the environment is facilitated by the shedding of a large number of oocysts by the host. Oocysts are resistant to environmental factors and disinfection [56]. Oocysts *excyst in the small bowel*, releasing *sporozoites* that *invade enterocytes.* Recipients of transplanted organs may become infected with *Cryptosporidium* spp. in three different ways: (i) transfer through transplantation, (ii) *de novo* infection, or (iii) reactivation of latent infection due to immunosuppression [57]. Zoonotic transmission of *Cryptosporidium* spp. infection cannot be excluded in transplant recipients due to exposure to socio-economic conditions. It is postulated that the transmission of oocysts may also occur through inhalation or accidental entry of oocysts into the respiratory tract during vomiting [28,58]. *Cryptosporidium* spp. may spread hematogenously from the gastrointestinal tract. The parasite has been detected in the colonic vascular system during histopathological examination [59]. The excretion of oocysts by infected individuals can exceed $<10^9$ oocysts in stool per day, with the excretion process lasting up to 50 days after the resolution of diarrhea symptoms.

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Host immune Response to *Cryptosporidium* Infection

Both cellular and humoral immunity play a role in *Cryptosporidium* spp. *infection* [60]. Both components of the immune response play a significant role in combating infection and acquiring immunity. The nature of the immune response in *Cryptosporidium* spp. infection is poorly understood. Much of the data is based on studies conducted using laboratory animals. However, a good experimental model has not yet been developed, and research is ongoing to establish such a model, including studies on various inbred strains of mice, including those with immunosuppression. Most commonly used for research are adult SCID mice and nu/nu mice of all strains, as they develop an initial level of immunity to *Cryptosporidium* spp. infection within 3 to 5 weeks post-infection [61]. Cell lines are also used for research purposes; however, to date, it has not been confirmed that the same parasite-host interactions occur in the intestinal mucosa in vitro and *in vivo*. Nevertheless, the occurrence of severe *Cryptosporidium* spp. infection in patients with reduced immunity and lymphocyte or gammaglobulin deficiencies may suggest the involvement of both cellular and humoral immune responses [62]. In innate immune defense, an initial mechanical and functional barrier involves the mucus layer of the small intestine and intestinal epithelial cells (IECs). Infection with *Cryptosporidium* spp. activates an intracellular signaling cascade. IECs, similar to biliary epithelial cells, express Toll-like receptors (TLRs), including TLR 2, 4, 5, and 9, which modulate the host immune response and subsequent parasite clearance [39,63–66]. Recognition and binding of *Cryptosporidium* spp. ligands by TLRs lead to the activation of a signaling cascade involving the myeloid differentiation primary response gene 88 (MyD88) adaptor protein and the activation of nuclear factor kappa B (NF-κB). Activation of NF-κB results in the expression of genes responsible for the production of chemokines, pro-inflammatory enzymes, cytokines, including tumor necrosis factor α (TNFα), interleukins (IL-1, IL-4, IL-8, and IL-15), C-C motif chemokine ligand 2 (CCL2), and C-X-C motif chemokine ligand 10 (CXCL10), which are responsible for recruiting inflammatory cells and activating acquired immune cells [67,68]. It has been observed that *Cryptosporidium* spp. infection initially reduces the production of human beta defensins 1 (HBD-1), which may facilitate parasite survival [69]. On the other hand, infection also induces the expression and secretion of HBD-2, which acts against the parasite in vitro and may play a role in recruiting T cells and dendritic cells *in vivo*, facilitating pathogen eradication [64]. IECs also secrete prostaglandins, which increase the secretion of intestinal fluids and antimicrobial peptides (AMPs), including α- and β-defensins and cathelicidins capable of directly killing sporozoites in vitro [69–71]. NF-κB activation leads to the production and release of chemokines and enhances the regulation of anti-apoptotic signals, such as Bcl-2 [72]. Although prostaglandins may contribute to diarrhea pathogenesis by altering chloride ion uptake and fluid secretion, they may also increase mucin secretion from epithelial cells, protecting against *Cryptosporidium* spp. infection by disrupting parasite adhesion. Additionally, prostaglandins may stimulate the production of certain HBDs and reduce the expression of pro-inflammatory cytokines. It is postulated that the predominant role in the immune response is played by the cellular response, particularly involving interferon γ (IFN-γ) participating in parasite clearance from the gastrointestinal tract and $CD4^+$ T cells [73]. It has been noted that the risk of *Cryptosporidium* spp. infection development is mainly associated with a decrease in $CD4^+$ T-cell count below 200 cells/μL [74]. In AIDS patients infected with *Cryptosporidium* spp., granulocytes are not the main component of the inflammatory response [75]. In AIDS patients, depletion of effector T lymphocytes may lead to increased parasite numbers, causing excessive production of CXCL-10. Effective antiretroviral therapy has been observed to be associated with reduced CXCL-10 production by enterocytes [76]. Reduced CXCL-10 levels may also decrease inflammatory reactions in the intestinal mucosa and lead to alleviation of chronic diarrhea. Potentially CXCR-3-positive cells are located in the epithelial layer; however, these cells apparently cannot eliminate the parasite. Effective antiretroviral therapy has been observed to restore the number of $CD4^+$ T lymphocytes in intestinal tissues. Humoral immunity also plays a significant role, as patients with primary immunodeficiency, including X-linked hyper-IgM syndrome and IgA deficiencies, are more susceptible to *Cryptosporidium* spp. [77]. In immunocompetent individuals after *Cryptosporidium* spp. infection, a humoral response of IgG, IgM, and IgA dependent on the number of CD4 cells develops [62]. However, the role of antibody

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responses in protection is still unclear. Based on serological studies in volunteer groups, individuals with antibodies against *Cryptosporidium* spp. were observed to have less severe symptoms of cryptosporidiosis upon subsequent pathogen exposure [78]. However, it is unknown whether antibodies provide protection against infection or are merely markers of the immune response [73].

Laboratory Diagnosis of *Cryptosporidium* spp. Infection

Cryptosporidiosis should be considered in cases of diarrhea of unknown epidemiology, especially in patients with lowered immunity levels. The material for examination is stool, and samples from the *respiratory tract, including* saliva, bronchoalveolar lavage (BAL), sputum, bronchial aspirate, and respiratory epithelial biopsy. Occasionally, colonic biopsies are used, which play an important role in the diagnosis and management of GI disease in renal transplant patients [79,80]. In some patients, mucosal biopsies from the intestine, as well as bile and other body fluids depending on symptoms, are utilized [81,82]. For the diagnosis of *Cryptosporidium* spp., fresh, frozen, and formalin-fixed stool samples are used. Stool samples fixed with polyvinyl alcohol (PVA) cannot be used in some staining techniques and formally for molecular diagnosis. Most laboratories routinely use microscopic methods in the diagnosis of cryptosporidiosis. Microscopic diagnosis is based on detecting the presence of *Cryptosporidium* spp. oocysts in samples of the examined material using light microscopy or phase-contrast microscopy. However, methods based on the evaluation of unstained preparations are associated with significant limitations due to the small size of oocysts (3-8 μm) and their similarity to other structures, including debris, yeast forms, and other protozoa. Additionally, a minimum threshold of 50,000 oocysts/ml in a stool sample is required for detection. Therefore, staining methods such as Kinyoun Modified Acid Fast, modified Ziehl-Neelsen method (Figure 1), and alternatively aniline-carbol-methyl violet (ACMV) staining are most commonly used. It has been observed that to increase sensitivity, multiple samples should be examined (Table 2).

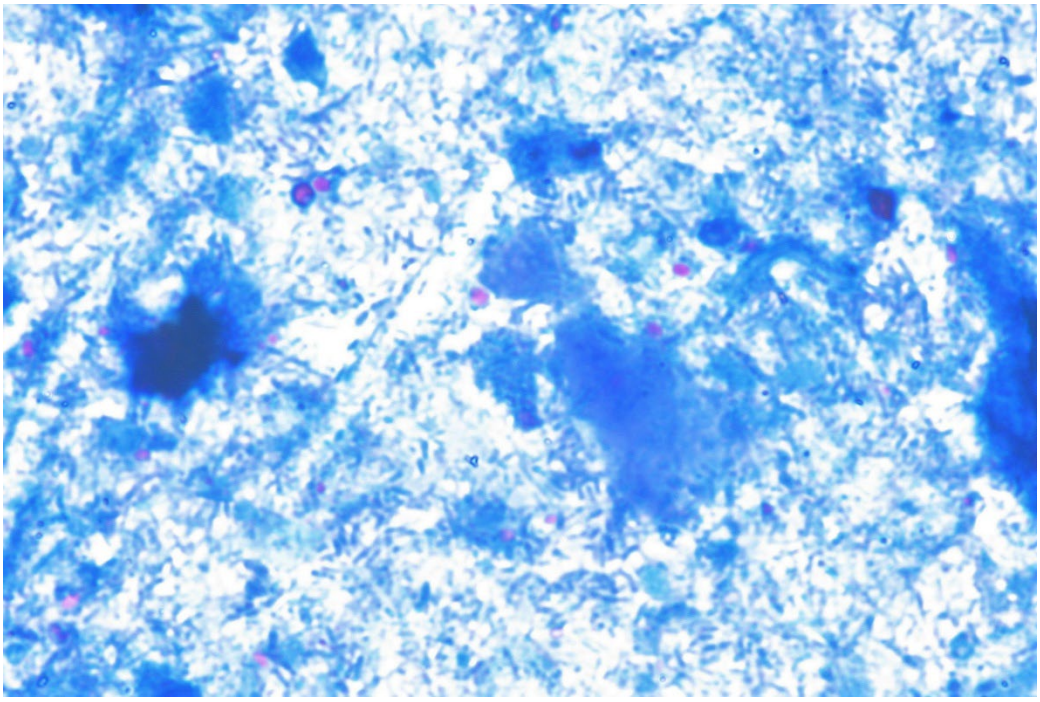

Figure 1. Oocysts of *Cryptosporidium* spp. in a direct smear (modified Ziehl–Neelsen staining, ×1000).

The sensitivity of this method is enhanced by concentrating oocysts in stool through centrifugation (1,200 x g), Sheather's sugar flotation method, saturated salt flotation, and Allen and Ridley's formalin-ether method [83]. However, even with the use of concentration methods, the sensitivity of the method largely depends on the skills of the person evaluating the preparation. Moreover, these procedures often may be insufficient to detect the presence of *Cryptosporidium* spp. oocysts in asymptomatic infections.

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Table 2. *Cryptosporidium* spp. infections in adult kidney transplant recipients (M-FECT, modified formalin-ether concentration technique; MAF, modified acid-fast method; ZN, Ziehl-Neelsen; MZN, modified Ziehl-Neelsen; NR – not reported).

Country	Number of participation		diagnostic method	patients with diarrhea	citation
	all (n)	infected (n/%)			
Argentina	26	11/42.3%	MAF after M-FECT	0	[25]
Türkiye	69	13/18.8%	MAF	8	[41]
Brazil	23	8/34.8%	ZN after FECT	-	[84]
India	60	12/20%	MAF	2	[85]
India	60	12/20%	MAF	16.6%	[86]
Iran	87	10/11.5%	MAF	-	[87]
Türkiye	43 transplant (40 renal and 3 liver)	7/21.2%	ZN	40	[88]
Pakistan	644	343/53%	MZN	343	[89]
Sudan	300	5/1.7%	NZN, MAF	ND	[90]
India	358	13/8.4%	MAF after FECT	-	[91]
France	307	30/9,8%	PCR	26	[92]
	88	41/46.6%		-	[92]
	73	6/8.2%	MZN	6	[3]

Immunofluorescence antibody staining (IFA) techniques using monoclonal antibodies against oocyst wall antigen are also used. They are characterized by high sensitivity and are cheaper compared to other traditional staining methods [93]. Serological methods, which rely on the detection of *Cryptosporidium* antigens or antibodies directed against this pathogen, are characterized by higher sensitivity and specificity than microscopic techniques [94]. Antigen detection can be performed using fluorescently or enzymatically labeled antibodies. Serological methods are considered the best tools for screening a large number of samples, especially in epidemiological studies. Coproantigen detection kits for *Cryptosporidium* spp. or in combination with *Giardia intestinalis* and/or *Entamoeba histolytica* are commonly used [95]. Detection of antibodies against *Cryptosporidium* spp. specific antigens in serum, saliva, or stool samples is an indirect diagnostic method. The detection of specific antibodies is beneficial only in cases of seroconversion, showing an increase in titer or a change in antibody isotype. Molecular methods are more sensitive, with a detection range from 1 to 106 oocysts. Polymerase chain reaction (PCR), quantitative Real-Time PCR (qRT-PCR), restriction fragment length polymorphism (PCR-RFLP), multiplex allele-specific-PCR (MAS-PCR), and quantitative real-time PCR are used. Genes such as 18S rRNA, TRAP C1, COWP, Hsp 70, and DHFR, subtype glycoprotein (GP) 60 gene, minisatellite, and microsatellite markers are used for species identification, as well as analysis of extrachromosomal double-stranded RNA elements [83]. Due to the structure of the oocyst wall, the DNA isolation procedure requires additional steps of initial homogenization, mechanical homogenization using glass beads, enzymatic lysis, alternate freezing and thawing of biological material, or incubation at temperatures above 70°C [93]. For species determination, the analysis of the rRNA small subunit locus by PCR RFLP is commonly used. Subtypes can be identified using PCR RFLP or sequencing of polymorphic loci, with the Cpgp40/15 locus being the most commonly used (also known as GP60) [68]. Fluorescent in situ hybridization (FISH), where oligonucleotide probes

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are used to detect the presence of 18S rRNA sequences, can also be applied for *Cryptosporidium* spp. detection. However, this method is time-consuming and less sensitive than molecular methods. Histological examination of intestinal mucosal biopsies is rarely used for routine diagnosis due to uneven parasite distribution in the biopsy, which can lead to false-negative results, and it is an expensive and time-consuming technique [83].

Treatment for *Cryptosporidium* Infection

Treatment for cryptosporidiosis, including in transplant recipients, are limited, which may be due to the absence of many organelles in *Cryptosporidium* spp., including the apicoplast, the target of many pharmacological therapies [96]. Various drugs are used in the treatment of cryptosporidiosis, including nitazoxanide (NTZ), paromomycin, trimethoprim/sulfamethoxazole, rifampin, and fluoroquinolones, either alone or in combination, with variable response durations [97] (Figure 1).

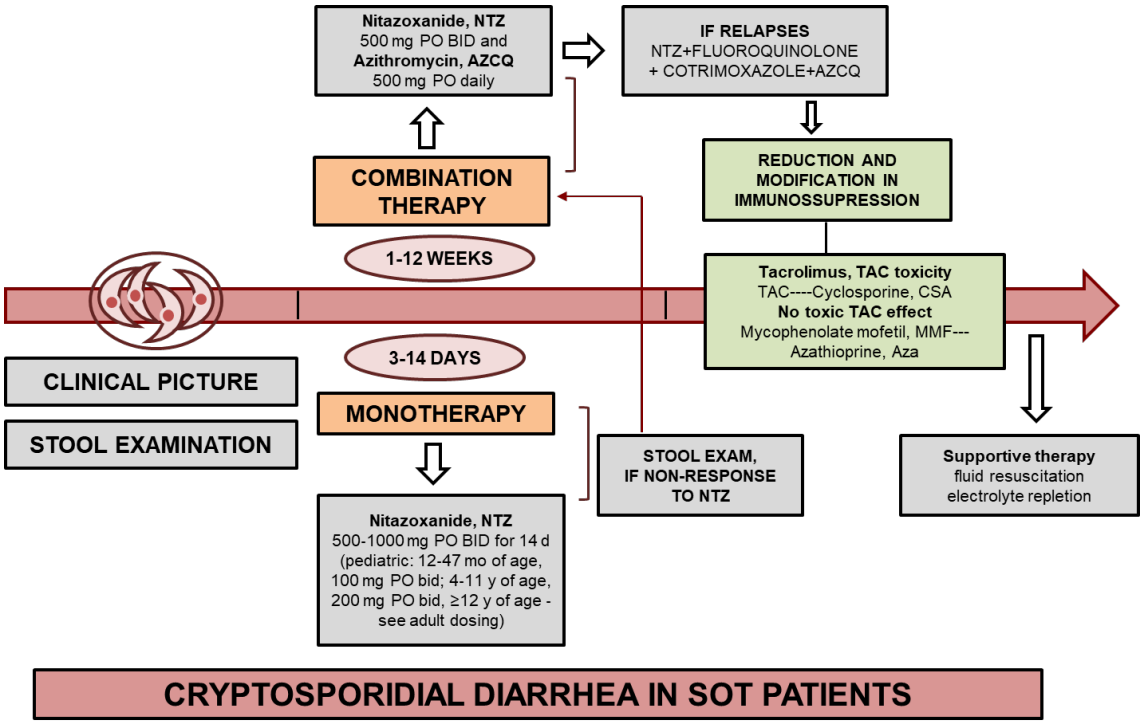

Figure 1. Treatment algorithm for *Cryptosporidium* sp. infection in patients after solid organ transplantation, SOT.

Nitazoxanide has been approved by the Food and Drug Administration (FDA) as the only drug for the treatment of diarrhea caused by *Cryptosporidium* spp. [98]. It inhibits the action of pyruvate ferredoxin oxidoreductase, an enzyme responsible for electron transport in anaerobic energy metabolism. This is probably not the only mechanism of its action. Based on randomized studies, it has been found that NTZ leads to faster resolution of symptoms and oocyst excretion [99,100]. The drug is available in 500 mg tablets or as a 100 mg/5 ml suspension; taking it with food increases its bioavailability. However, the effectiveness of NTZ in the treatment of cryptosporidiosis in immunosuppressed patients has not been established, which is likely due to the low number of $CD4^+$ lymphocytes [101]. In adult HIV-infected patients, NTZ has not been shown to affect the duration of diarrhea and oocyst excretion [44]. Therefore, it has not been approved by the FDA for the treatment of *Cryptosporidium* spp.-induced diarrhea in HIV patients. For solid organ transplant (SOT) recipients, the American Society of Transplantation Infectious Diseases Community of Practice recommends the use of NTZ in the treatment of cryptosporidiosis. Data on the use of NTZ in transplant recipients are limited and mainly come from case reports. However, some researchers have noted that long-term NTZ therapy is often effective [102,103]. In immunocompetent patients, the duration of NTZ

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treatment is three days, while in patients with decreased immunity, including kidney transplant recipients, it can be longer, up to 14 days [104]. Tomczak et al. [44] noted that in SOT patients, resolution of diarrhea was achieved by combining high-dose NTZ with rifaximin and azithromycin (AZCQ), along with reducing tacrolimus levels. Additionally, some studies have shown that macrolides, including AZCQ and spiramycin, have activity against *Cryptosporidium* and have shown promising results in transplant recipients [105]. Adjusting immunosuppression and monitoring immunosuppressant levels is crucial in both the management and prevention of cryptosporidiosis. Tie et al. [105] observed in a patient after liver transplantation that NTZ therapy, combined with controlled $CD4^+$ T cells at 100-300/mm^3, was highly effective against *Cryptosporidium* without inducing immunorejection. The American Society of Transplantation Infectious Diseases Community of Practice recommends both reducing immunosuppression and using NTZ as initial treatment for SOT recipients with cryptosporidiosis. It has been noted that the severity of the disease probably depends on the degree of immunosuppression and the number of $CD4^+$ T cells, so it is very important in SOT patients to attempt to restore immune function by adjusting or changing immunosuppressive therapy [97]. A higher frequency of *Cryptosporidium* spp. infection has been demonstrated in individuals taking tacrolimus compared to cyclosporine. It has also been found that diarrhea caused by *Cryptosporidium* spp. may lead to increased tacrolimus levels, which may in turn worsen kidney function and prolong immunosuppression [35,36,104].It has been found that mycophenolate mofetil (MMF) may have activity against *Cryptosporidium* spp. by inhibiting folate metabolism [102]. In treatment, conversion to a less potent immunosuppressive drug (cyclosporine) or reduction, if necessary, of previously administered immunosuppressive drug doses should be considered [35]. Infection of the biliary tract in immunocompromised patients may constitute an extraintestinal reservoir, leading to a lack of response to certain drugs (paromomycin) and may lead to relapses. In these patients, drugs excreted in bile, including NTZ, should be administered [106]. Oral bovine immunoglobulin (hyperimmune colostrum) appears to be a viable alternative treatment for cryptosporidiosis [107]. Additionally, a great promise is shown by calcium-dependent protein kinases, microtubule formation inhibitors, hexokinase, lactate dehydrogenase, inosine-5-monophosphate dehydrogenase, and fatty acyl-coenzyme A binding inhibitors, as well as anti-parasite vaccines [96,108]. Love et al. [96] observed that clofazimine also exhibited efficacy against *Cryptosporidium*, making it a potentially new cryptosporidiosis treatment and a novel chemical tool for understanding *Cryptosporidium* biology. Kabir et al. [109] suggested that the compounds atropine sulfate and bufotalin could be useful in the development of new anti-*Cryptosporidium* medications. Symptomatic treatment should also be considered, including correction of fluid and electrolyte deficits, and appropriate nutrition. Garlic (*Allium sativum*) has an anti-parasitic effect against many parasites [110]. The effect of zinc oxide nanoparticles (ZnO-NPs) as carriers for garlic and NTZ has been investigated in animal models. In well-documented studies, ZnO-NPs have been used as an effective alternative therapy in the treatment of experimental cryptosporidiosis, especially in combination with other treatment methods that enhance their antioxidant activity [111,112]. Research is being conducted on the use of nanoparticles with specific anti-protozoal medications to treat cryptosporidiosis [113].

Prevention

Firstly, to eliminate the possibility of nosocomial infection in patients who will often be in contact during consultations in the nephrology department; secondly, to determine the source of contamination (notably animal) and eliminate it to prevent any subsequent recontamination. To minimize the risk of infection, handwashing is recommended, as well as avoiding contact with young pets and farm animals (calves), infected individuals, and swimming pools. It is also recommended to protect against waterborne *Cryptosporidium* spp. infections, including boiling drinking water and filtering water through filters that trap particles around 1 μm in size. Pasteurization of milk for approximately 15 seconds at a temperature of around 70°C is also recommended, as it destroys oocysts.

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Conclusions

Cryptosporidium spp. infection should be considered in kidney transplant patients with diarrhea. Diagnostic methods should include direct methods and, if possible, molecular methods. Treatment of cryptosporidiosis is combination therapy using nitazoxanide, azithromycin, and rifaximin.

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