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Cryptosporidiose et microsporidiose responsables de diarrhée chez le patient transplanté rein et/ou pancréas

Cryptosporidiosis and microsporidiosis as causes of diarrhea in kidney and/or pancreas transplant recipients

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Contribution of authors

Clément Deltombe: author, designed the study, wrote the article.

Maeva Lefebvre: author, designed the study, reviewed the article.

Florent Morio: author, designed the study, reviewed the article.

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Résumé

Introduction. Les troubles gastro-intestinaux chez les patients transplantés d'organes solides (TOS) peuvent avoir des origines multiples, dont la cryptosporidiose et la microsporidiose, bien que leur prévalence et incidence soient encore mal connues dans les pays développés.

Patients et méthodes. Nous avons évalué de manière prospective les causes infectieuses de diarrhées chez les patients TOS. Les objectifs secondaires de l'étude étaient de décrire les cas de cryptosporidiose et d'en rechercher les facteurs de risque. Tous les patients adultes transplantés rein et/ou pancréas souffrant de diarrhée et vus en consultation ou admis en hospitalisation entre le 01/05/2014 et le 30/06/2015 ont été inclus. Un examen de selles a été réalisé selon un protocole standardisé incluant une recherche bactériologique, virologique et parasitologique. Les données clinico-biologiques concernant les symptômes, l'immunosuppression et les facteurs de risque environnementaux étaient issues d'un auto-questionnaire et du dossier médical informatisé.

Résultats. Parmi les 73 patients inclus, 36 avaient une diarrhée infectieuse (49,3 %). Les causes les plus fréquentes étaient virales (17/36) et parasitaires (11/36), avec au premier rang la cryptosporidiose (n=6). De façon plus inattendue, les microsporidioses arrivaient au 3^{e} rang (n=4). La prévalence estimée de la cryptosporidiose et de la microsporidiose dans cette cohorte était de 3,7 et 2,4 $^{0}/_{00,}$ respectivement. Aucun facteur de risque spécifique, immunologique ou environnemental, n'a pu être mis en évidence.

Conclusion. La cryptosporidiose et la microsporidiose représentent une cause significative et possiblement sous-estimée de diarrhée chez le patient TOS en France.

Abstract

Introduction. Gastrointestinal disorders in solid organ recipients may have various origins including cryptosporidiosis and microsporidiosis. The prevalence of these infections is poorly known in solid organ transplant (SOT) patients in industrialized countries.

Methods. We prospectively assessed the infectious causes of diarrhea in SOT patients. Secondary objectives were to gain further insight into the main characteristics of cryptosporidiosis, and to assess risk factors for this infection. All adult kidney and/or pancreas recipients presenting with diarrhea and admitted to our facility between May 1, 2014 and June 30, 2015 were enrolled. A stool sample was analyzed using a standardized protocol including bacteriological, virological, and parasitological investigations. Data related to clinical symptoms, immunosuppression, and environmental potential risk factors were collected through a self-administered questionnaire and computerized medical records.

Results. Out of 73 enrolled patients, 36 had infectious diarrhea (49.3%). Viruses ranked first (17/36), followed by parasites and fungi (11/17). Cryptosporidiosis was the most common parasitic disease (n=6 patients). We observed four microsporidiosis cases. The estimated prevalence of cryptosporidiosis and microsporidiosis in this cohort was 3.7 and $2.4^0/_{00}$, respectively. No significant risk factor for cryptosporidiosis or microsporidiosis, neither environmental nor immunological, could be evidenced.

Conclusion. Both cryptosporidiosis and microsporidiosis represent a significant cause of diarrhea in kidney transplant recipients.

Introduction

Digestive disorders are a common complaint in solid organ transplant (SOT) patients [1]. They are frequently considered as drug adverse effects, mainly due to mycophenolic acid, but they can also be associated with opportunistic infections as the result of drug-induced over-immunosuppression. Recent data shows that infectious agents could be responsible for up to 50% of diarrhea episodes in kidney transplant recipients [2].

Cryptosporidiosis occurs worldwide and is considered the second leading cause of diarrhea after rotavirus infection [3]. Although its prevalence is relatively low in high-income countries [4,5], the disease can be responsible for massive epidemics as illustrated in Milwaukee in the 1990s (400,000 cases), in France in 2001, or in Sweden in 2011 [6–8]. It has also been reported in HIV-infected patients. However, in solid organ transplantation, limited data is available regarding the incidence of the disease, its characteristics, and relation with the degree of immunodeficiency [9].

Microsporidia are obligate intracellular ubiquitous pathogens, related to fungi and responsible for severe diarrhea in immunocompromised patients among whom HIV patients [10]. However, in the last years, an increased number of cases have been reported in patients undergoing solid organ transplantation [11]. *Cryptosporidium* sp. and microsporidia are neglected pathogens likely underdiagnosed in immunocompromised patients as both require specific diagnostic methods and most physicians are not aware of these diseases.

The main objective of the study was to assess infectious causes of diarrhea episodes observed over a 1.5-year period in a reference center for kidney/pancreas transplantation, with a focus on both cryptosporidiosis and microsporidiosis.

Patients and methods

Study design

Between January 2014 and June 2015, all adult recipients of a kidney and/or pancreas transplant attending the nephrology outpatient or hospitalization department of Nantes University Hospital (Western France) and declaring having diarrhea were prospectively enrolled and submitted to a complete microbiological investigation to discriminate between drug-induced and infection-induced etiologies.

Diarrhea was defined as the emission of loose or liquid stools more than three times per day or at an abnormal frequency. Patients with known chronic intestinal disease or any surgery with resection of part of the intestine or carrying an ostomy pouch were excluded. The management of patients, irrespective of the cause of diarrhea, was left to the discretion of the transplant physician. As part of our standard protocol, all patients received co-trimoxazole for 6 months to prevent *Pneumocystis jirovecii* pneumonia and valganciclovir to prevent cytomegalovirus (CMV) infection for 3 or 6 months (according to their CMV status on the day of transplantation). Patients were followed up for 6 months after inclusion. The study was approved by the local ethics committee (number 2014-04-03). Patients were informed and were asked to give signed consent.

Microbiological investigations

Every patient's stool sample collected at inclusion was investigated using a standardized protocol including viral, bacterial, and parasitological investigations including microsporidia. Investigation for viral infections included: *i)* detection of adeno- and rotaviruses by immunochromatography (Rapid Strip Rota-Adeno by Meridian^{TM}) and PCR for identification of the 40-41 most frequent serotypes among adenoviruses; *ii)* PCR on stool for astro- and

noroviruses; *iii)* blood PCR for CMV [13]. Bacteriological investigation consisted of standard stool cultures. *Clostridium difficile* infection was ruled out by the glutamate dehydrogenase test followed, if positive, by a cytotoxicity test or a toxin test. Parasitological investigation included: *i)* direct microscopic examination after iodine-stained wet mount and following a Bailenger's biphasic concentration method, *ii)* specific detection of *Cryptosporidium* oocysts using the modified Ziehl-Neelsen staining, and *iii)* specific detection of *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* using two in-house specific real-time PCR assays [12,13].

Immunosuppression and environmental risk factors

Demographic and clinical data was extracted from the patients' medical records (Divat cohort, approved by the French National Commission on Computing and Liberty DR- 2025-087, number 914184; February 15, 2015). The following variables were recorded: level of immunosuppression according to the type of transplantation, induction treatment, number of immunosuppressive drugs, plasma level of calcineurin inhibitors at inclusion, CD4 lymphocyte count at inclusion, and last available gamma globulin dosage (checked routinely once a year). We also recorded all immunological events that led to an increased immunosuppression as well as clinical manifestations associated with over-immunosuppression, such as BK virus, CMV replication and disease, or *Pneumocystis jirovecii* pneumonia.

For each episode of diarrhea, patients filled a self-administered questionnaire including characteristics of diarrhea (duration, number of stools per day), associated symptoms (weight loss, low urine production, fever/chills, abdominal pain, nausea/vomiting, asthenia, headache), and data about their lifestyle. All items covered most of the known risk factors for cryptosporidiosis in the previous six months, allowing gaining further insights into the

possible source of contamination. Detailed information about contact with water either used for drinking (mineral, tap water, or untreated), for bathing (swimming pool, river, etc.), recent history of travel abroad, as well as contact with animals or young children or anyone with diarrhea were investigated. Professional activities were also stratified according to the risk of cryptosporidiosis: activities such as contact with animals or biological fluids were considered as high risk; professions with manual activities or with frequent physical contact with people were considered as medium risk, and retired patients or people with solitary professions were considered at low risk.

Statistical analysis

Statistical analyses were performed with the SAS software (version 9.2). Quantitative parameters were compared using Student's *t* test in cases of normal distribution, otherwise using the non-parametric Wilcoxon-Mann-Whitney test. Factors associated with infectious diarrhea, and cryptosporidiosis were studied in a univariate analysis: qualitative parameters were compared using the Chi2 test when the subjects were greater than or equal to 5 and the Fisher test if not. Only comparisons between independent groups have been performed in the study. A 5% alpha error value was considered significant.

Results

Results of the microbiological tests

During the 12 months of the study, 73 patients were enrolled in this prospective study (four were excluded due to the impossibility of collecting stool samples). Seven patients experienced two episodes of diarrhea during the study period. The main demographic and clinical characteristics are summarized in Table I. For all patients, the immunosuppressive

maintenance therapy post-transplantation combined a calcineurin inhibitor and mycophenolic mofetil.

Infectious diarrhea was confirmed in 31 (42.4%) of the 73 patients (Figure 1). A wide range of gastro-intestinal pathogens were identified among which viruses ranked first (17/31, 59.4%), followed by parasites-fungi (11/31, 35.5%), and bacteria (5/31, 16.1%). *Cryptosporidium* spp. was the most common parasite in this cohort (n=6) whereas a single patient was infected by *Giardia intestinalis*. Interestingly, *Enterocytozoon bieneusi* (microsporidia) was the cause of diarrhea in four patients. Cryptosporidiosis and microsporidiosis respectively represented 8.2% and 5.5% of patients with diarrhea. Over this period, as 1,624 kidney and/or pancreas transplant recipients were followed in our center, the minimum incidence of cryptosporidiosis and microsporidiosis could be estimated at $3.7^{0}/_{00}$ and $2.4^{0}/_{00}$, respectively. Coinfections were identified in only two patients (6.5%), one with norovirus/*C. difficile* and one with *Cryptosporidium* sp./norovirus infection.

Description of patients with cryptosporidiosis

Characteristics of the six patients with cryptosporidiosis are detailed in Table II. The median age was 58.5 years (mean: 56.8; interquartile range [IQR] 25-75 [44.75;77]). Cryptosporidiosis occurred at a mean time of 33.9 months (median: 40.5 months; IQR 25-75 [6.25;65]) following transplantation although much earlier in two patients (Day 2 and Day 14, respectively), suggesting that they were probably infected, but asymptomatic, prior to transplantation. At inclusion, patients with cryptosporidiosis had been experiencing diarrhea for a mean time of 25.3 days (min 2 days, max 87 days, IQR 25-75 [7.25;87]). Three patients presented with fever, three with vomiting, and three with abdominal pain. All patients presented with dehydration and a mean weight loss of 3.6 +/- 2.4 kg. Acute transplant dysfunction was evidenced in two patients. *Cryptosporidium parvum* was identified in four

patients and *Cryptosporidium felis* in one patient who owned a cat (species identification was not possible in the remaining patient). None of these patients had a previous history of cryptosporidiosis.

In all patients, mycophenolic mofetil was reduced or stopped until diarrhea resolved. Symptomatic treatment alone was sufficient in three patients and gastrointestinal disorders resolved within two weeks. The others were given 500 mg of nitazoxanide twice a day for four weeks. We implemented a long treatment period as we had observed relapses following shorter treatment regimens in previous patients. Patients were followed up in an outpatient clinic every 15 days during the treatment period. Gastrointestinal disorders resolved in the first two weeks for the three patients. Mycophenolic mofetil was newly initiated as soon as diarrhea resolved. No relapse was observed. One patient had diarrhea recurrence due to a *Campylobacter* sp. infection, and another was found deceased at home from a probable cardiovascular cause five months after the cryptosporidiosis episode.

Risk factors for cryptosporidiosis

We analyzed the level of immunosuppression and the presence of environmental risk factors in the patients with cryptosporidiosis. Two had a history of diabetes and one of neoplasia. All had been dialyzed before transplantation and all but one were recipients of a first kidney transplant. One underwent a desensitization protocol before transplantation (with plasmapheresis, polyvalent immunoglobulin, and rituximab). Two patients received a lymphocyte depleting induction therapy with thymoglobulin, four with basiliximab (anti-interleukin-2 receptor). Maintenance therapy consisted of calcineurin inhibitors and mycophenolic mofetil in all patients with cryptosporidiosis. One had been treated for two acute rejections. One had a history of cytomegalovirus infection and two had BK virus

infection. The mean CD4 lymphocyte count was 484 (IQR 25-75 [155;956]) and mean gamma-globulin value was 9.9 (IQR 25-75 [8.6;16]).

Data generated using the self-administered questionnaire on environmental risk factors highlighted that among the six patients with cryptosporidiosis, only one had no risk factor. In the five other patients, we observed contact with recreative water or untreated water (n=3), recent antibiotic therapy (n=3), daily contact with animals (n=2), contact with potentially infected persons (n=2), and unwashed vegetable consumption (n=1).

By comparison, among the 25 patients with another cause of infectious diarrhea, 19 had been dialyzed, and eight of them (32%) had undergone a second (or more) transplantation. Induction treatment was a lymphocyte-depleting therapy for 13 (52%) patients and a non-depleting therapy for 10 (40%) patients (two patients had no induction therapy). All patients were treated with tacrolimus and mycophenolic mofetil as maintenance therapy. Four patients experienced acute rejection. CMV and BK virus infection occurred in five patients and one patient, respectively. The mean CD4 lymphocyte count was 411/mm^3 +/- 325. The mean gamma globulin dosage was 7.9 g/L +/- 2.8. Thus, no difference was observed between infected patients with or without cryptosporidiosis.

Microsporidiosis as an alternative cause of diarrhea among kidney transplant recipients

We identified intestinal microsporidiosis due to *Enterocytozoon bieneusi* in four patients of the prospective cohort (4 out of 31 patients with infectious diarrhea, 13%). One patient had not been dialyzed before transplantation. Two patients had diabetes and one had neoplasia. Except for one patient (3^{rd} transplantation), all patients experienced their first transplantation. Most patients had received non-depleting induction therapy (n=3). All were treated using calcineurin inhibitors and mycophenolic mofetil. Half of them had a history of

CMV infection (none infected with BK virus). Microsporidiosis occurred after a mean of 28 months post transplantation (min 2 months, max 73 months, IQR 25-75 [10.25;73]). Clinical characteristics of these patients are detailed in Table III. Mean CD4 T cell counts at the time of diagnosis were 842/mm^3 (min 500/mm^3, max 1,100/mm^3; IQR 25-75 [614;1,100] SD+/- 312/mm^3), mean gamma globulin level was 8.4 g/L (min 4.3 g/L, max 15 g/L; IQR 25-75 [5.05;15] SD +/- 4.8 g/L), and the mean tacrolimus residual value was 7.6 ng/mL (min 4.1 ng/mL, max 13.0 ng/mL; IQR 25-75 [4.4;13.3] SD +/- 4.3 ng/mL) (objective: 6-8 ng/mL). Two patients were treated with albendazole (400 mg twice daily for 3 weeks) while diarrhea resolved spontaneously before initiating therapy for the two others. No relapse was observed in the following six months. Environmental risks were observed in only one patient with consumption of unwashed vegetables.

Discussion

Diarrhea is a very common complaint after solid organ transplantation [14]. The gastro-intestinal adverse effects of mycophenolic mofetil are well known and could possibly limit compliance with the immunosuppressive regimen [15]. In cases of severe diarrhea, the medication is usually stopped with potential immunological consequences such as graft rejection. Only a few studies analyzed the causes of diarrhea in adult kidney transplant patients, and all highlighted that infections could account for up to 50% of cases [2,16,17].

Prior to this prospective study we diagnosed five cases of cryptosporidiosis over five years at our facility. This prompted us to systematically investigate the causative agents of diarrhea in our patients, and resulted in the diagnosis of six additional cases over one year in the present study.

In our prospective series of 73 patients included over a 12-month period, a complete microbial screening allowed us to conclude that 42.4% (n=31) were of infectious origin and

mainly viral. Interestingly and quite unexpectedly, parasites ranked as the second highest cause of diarrhea after viruses (11/73, 15.1%), involving *Cryptosporidium* spp. and *E. bieneusi*. *Cryptosporidium* spp. was found in six patients (6/73, 8.2%), which corresponds to an estimated minimum prevalence of $3.7^{0}/_{00}$ with *C. parvum* as the main species. One patient was infected with *C. felis* suggesting horizontal transmission by his own cat.

Unfortunately, due to the small size of the cohort, we were not able to find statistically significant differences in clinical characteristics between patients with or without cryptosporidiosis. Nevertheless, some findings may be highlighted. The mean delay of infection onset was 40.5 and 28 months post-transplantation for *Cryptosporidium* sp. and *E. bieneusi*, respectively. It has already been suggested that diarrhea associated with immunosuppressive drugs are more likely to be observed early post-transplant when patients are administered many medications, often at higher doses, while infectious diarrhea occurs after several years [15]. As a consequence, diarrhea in the late post-transplantation period should not be underestimated and may lead to complete microbial investigation including cryptosporidiosis and microsporidiosis that require appropriate diagnostic methods. Surprisingly, two of our patients were diagnosed with cryptosporidiosis before day 14 post-transplantation. As both patients were still hospitalized when the diagnosis was made, it is therefore likely that both were already infected before admission and transplantation but asymptomatic. Indeed, it has been reported that *Cryptosporidium* oocysts can be present in the stools of 1% of immunocompetent persons in high-income countries [18]. The number should be higher in dialyzed patients who present with immunodeficiency and in whom diarrhea is also frequent [19].

Two studies having prospectively or retrospectively reviewed all their patients with diarrhea for cryptosporidiosis reported contrasting results, with no cryptosporidiosis observed in the

former in the United States [2,4] compared with up to 28% in the Indian cohort [20]. Recently, another study conducted in France and focusing on kidney transplantation identified cryptosporidiosis in seven of 64 pediatric recipients (11%) recruited over a three-year period [21]. These studies highlight that the prevalence of cryptosporidiosis probably varies greatly from center to center possibly depending on environmental factors and/or diagnostic strategy.

Laboratory diagnostic methods are also of major importance, as the detection of *Cryptosporidium* oocysts and microsporidia requires specific techniques (such as Ziehl-Neelsen staining or PCR assays). As a consequence, insufficient awareness of the pathogenic potential of these species, inappropriate diagnostic strategy, and/or insufficiently trained microscopists can lead to underestimating the prevalence of these opportunistic pathogens [22,23].

Our study also aimed at finding risk factors for cryptosporidiosis and microsporidiosis. Several parameters were selected as reflecting the level of immunosuppression: induction and maintenance immunosuppressive treatment, pre- and post-transplant over-immunosuppression (desensitization, acute rejection), infections such as CMV and BKV, CD4+ cell count and gamma globulin dosage. No difference was observed between patients with cryptosporidiosis or microsporidiosis and patients with diarrhea of another infectious origin. The environmental survey was also not successful at identifying specific risk factors.

The ANOFEL *Cryptosporidium* network (France) recently reported 47 solid organ recipients (41 kidneys) presenting with cryptosporidiosis between 2006 and 2010 and found environmental risk factors in 18 patients. Immunosuppression was higher in five patients who also had CD40 ligand deficiency (N=1) or HIV infection (N=4). Thirty-five patients were treated, six relapsed, and one died with a progressive infection [24]. Although this study also

reflects the importance of cryptosporidiosis in SOT patients, its retrospective design, without standardized questionnaire nor microbiological investigations could not estimate the prevalence of the disease.

The treatment of cryptosporidiosis is mainly based on the reduction of immunosuppression, as only few antiparasitic drugs are available and display only moderate efficacy [5,25]. In our series, nitazoxanide was administered to three patients over several weeks (instead of three days as usually recommended) because we previously observed recurrence in five patients treated for only three days. To our knowledge no randomized study was conducted in solid organ transplant recipients with cryptosporidiosis, and most knowledge was extrapolated from either data from immunocompetent hosts or patients with HIV infection [26].

In addition, we also reported that *E. bieneusi* microsporidiosis was responsible for diarrhea in up to 5.5% of our cohort (13% of patients with infectious diarrhea). Until now, microsporidiosis among kidney transplant recipients has been mostly limited to individual case reports [27]. Our prevalence is in stark contrast with recent data published by Echenique in the United States [4] in which no microsporidiosis was diagnosed in a retrospective study. This difference may be explained by the use of DNA detection by PCR in our study that is more sensitive than microscopy.

Despite being a prospective evaluation, our study had some limitations: *i)* potential patient memory bias exists, as patients self-reported their symptoms and risk factors for cryptosporidiosis, although this questionnaire was validated prior to the study; the study was limited to patients who were admitted and had a complaint of diarrhea. It is possible that other patients, with less severe manifestations, did not consult the nephrology department; *ii)* we only analyzed one stool sample which could have led to an underestimate of the prevalence of parasites, as three consecutive stool parasite examinations are usually

recommended, meaning that the prevalence of parasites could be even higher than observed.

The management of transplanted patients with diarrhea still remains difficult. The DIDACT study conclusions attempted to argue against the systematic change in immunosuppression that could have immunological consequences [2]. Fifty per cent of the episodes resolved after treatment of infection and withdrawal of immunosuppressive drugs. Whilst our study reported the same percentage of infectious diarrhea cases, we conclude that microbiological investigation of stools, although not always currently performed in routine practice, should be conducted prior to modification of immunosuppressive regimens.

Conclusion

Gastro-intestinal disorders remain a frequent complaint in kidney/pancreas transplant recipients and should be investigated. We described six cases of *Cryptosporidium* sp. infection in kidney and/or pancreas transplant recipients with a prospectively assessed prevalence of 3.7‰ among SOT patients with diarrhea. Surprisingly, we identified four cases of microsporidiosis, a disease still only poorly investigated in this population. These pathogens cannot be identified without a complete parasitological examination of the patient's stools. This should be recommended for transplanted patients even in developed countries. Taken together, our data strengthens the argument for routine clinical screening for these pathogens in cases of diarrhea occurring in high risk patients such as kidney/pancreas transplant recipients.

Disclosure of interests

The authors declare no conflict of interests.

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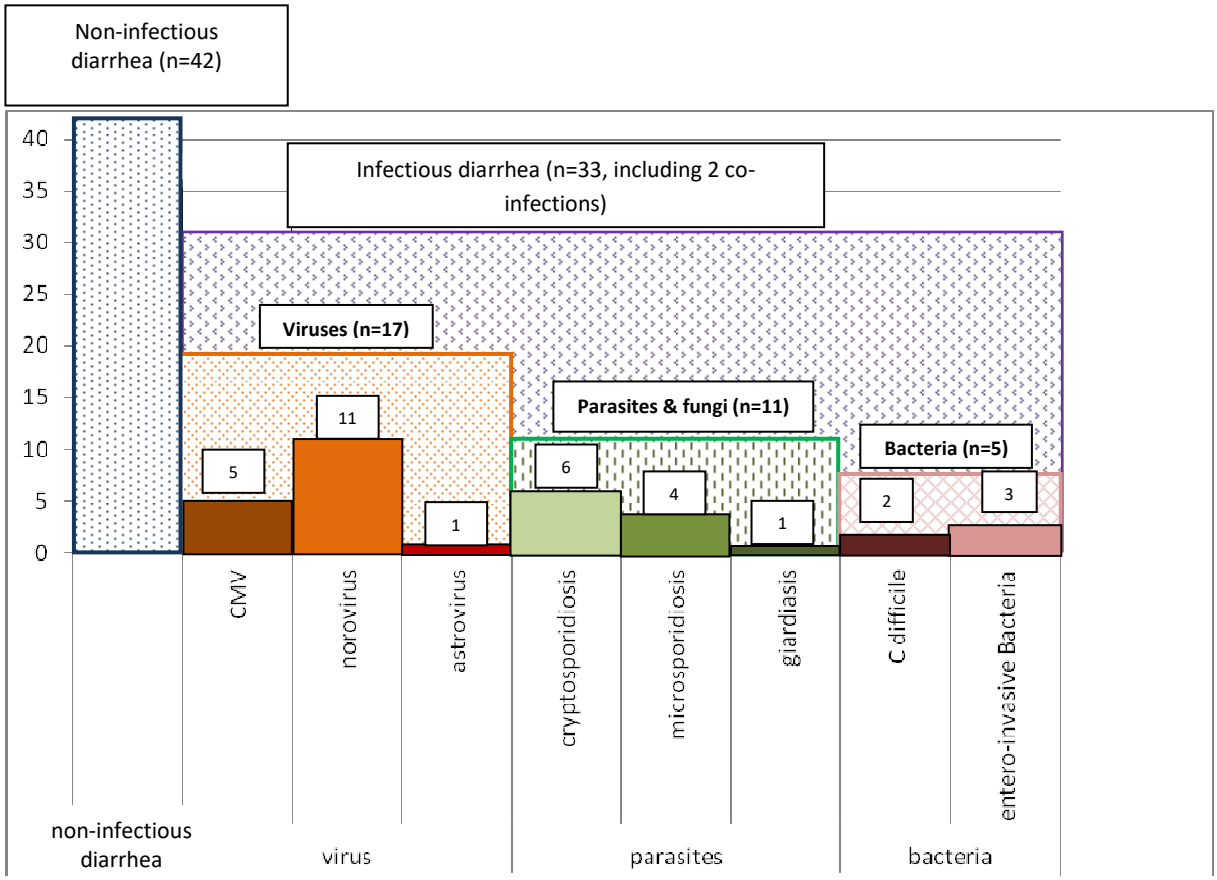

Figure 1. Etiological distribution of diarrhea (n=73 patients)

Figure 1. Répartition étiologique des diarrhées (N=73)

Table I. Main demographic and clinical characteristics of the enrolled cohort (n=73).

Tableau I. Principales caractéristiques démographiques et cliniques de la cohorte (N=73).

Item	Data
Demography	
Sex ratio (M/F)	1.43 (43/30)
Median age (years) +/-SD, [IQR] (extremes)	57 +/- 13.1 [44.25;63.75] (28-78)
Type of graft	Kidney: N=65 (89.1%) Kidney/pancreas: N=8 (10.9%)
Immunology	
Number of transplantations	≤1: N=61 (83.6%) >1: N=12 (16.4%)
Renal replacement therapy before transplantation	Hemodialysis: N=45 (62%) Peritoneal dialysis: N=10 (13%) None: N=18 (25%)
Induction therapy	Lymphocyte depleting: N=33 (45.2%) Lymphocyte non-depleting: N=36 (49.5%) none: N=4 (5.5%)
Median time since transplantation (months) +/-SD, [IQR] (extremes)	25 +/- 58.6 [3.25;66.5] (1-321)

Acute rejection	N=9 (12.3%)
Lymphocyte CD4+ mean rate +/-SD [IQR]	457 +/- 328/mm^3 [188;671] Median: 404/mm^3
Gamma globulin median rate +/- SD [IQR]	7.9 +/-3.1 g/L [6;9.7]
Tacrolimus median rate [IQR]	7.9 +/-4.8 ng/mL [6.1;10.2]
HIV infection	N=0 (0%)
Occupational risk	
Low	57 (78.1%)
Medium	11 (15.1%)
High	5 (6.8%)
Usual drinking water	
Tap water	32 (43.8%)
Mineral water	40 (54.8%)
Untreated water	1 (1.4%)
Living place (according to INSEE)	
Rural	28 (38.4%)
Peri-rural	7 (9.5%)
Peri-urban	10 (13.7%)
Urban	28 (38.4%)
Season at inclusion	
Autumn	16 (21.9%)
Winter	19 (26%)

Spring	18 (24.6%)
Summer	20 (27.4%)
Regular contact with animals	
No	38 (52.1%)
Yes	35 (47.9%)
Traveling abroad	
No	67 (91.8%)
Yes	6 (8.2%)
Contact with water	
None	50 (68.5%)
Swimming pool	13 (17.8%)
Untreated water	10 (13.7%)
Consumption of unwashed vegetables or washed with untreated water	
No	55 (75.3%)
Yes	18 (24.7%)
Contact with someone experiencing diarrhea	
No	61 (83.6%)
Yes	10 (13.7%)
Not known	2 (2.7%)
Did you help a child aged <5 years to go to the toilets in the previous 15 days?	
No	69 (94.5%)
Yes	4 (5.5%)

Do you have contact with children from nursery	
No	68 (93.2%)
Yes	5 (6.8%)
Antibiotics in the previous 3 months	
Missing data	1
No	41 (56.9%)
Yes	30 (41.7%)
Not known	1 (1.4%)
Co-trimoxazole treatment in the previous 3 months	
No	52 (71.2%)
Yes	21 (28.8%)

INSEE: French National Institute for Statistics and Economic Studies; [IQR]: interquartile range 25-75%

Table II. Clinical description and epidemiological characteristics of patients with cryptosporidiosis (N=6)

Tableau II. Description clinique et caractéristiques épidémiologiques des patients avec une cryptosporidiose (N=6)

Sex, age number of graft	**Season of infection**	**Time since transplantation**	**Induction and maintenance immuno-suppressive therapies**	**Symptoms**	**Time to diagnosis**	**Species**	**Co-infection**	**Environmental risk factor**	**Living place**
M, 68 y.o 1st graft	Summer	56 months	Anti-IL2r CNI + MMF	Diarrhea, vomiting, dehydration, weight loss (8 kg), acute kidney injury, acidosis	17 days	*C. parvum*	No	Contact with animals and children <5 y.o, untreated water, unwashed vegetables	Rural
F, 42 y.o 1st graft	Summer	25 months	Anti-IL2r CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (4 kg)	24 days	Non typed	No	Previous antibiotic therapy with amoxicillin-clavulanic acid	Rural
M, 77 y.o 1st graft	Summer	14 days	Anti-IL2r CNI + MMF	Severe diarrhea, dehydration, weight loss	3 days	*C. parvum*	No	Contact with untreated water	Peri-urban

				(3 kg), acute kidney injury					
M, 53 y.o 1st graft	Summer	2 days	Anti-IL2r CNI + MMF	Diarrhea, vomiting, dehydration, weight loss (4 kg)	2 days	*C. felis*	No	Contact with his cat, previous antibiotic therapy with quinolone	Urban
F, 64 y.o 1st graft	Autumn	65 months	Depleting therapy CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (2 kg)	19 days	*C. parvum*	No	None	Rural
F, 37 y.o 3rd graft	Autumn	57 months	Desensitization, depleting therapy CNI + MMF	Fever, abdominal pain, diarrhea, vomiting, dehydration, weight loss (3 kg)	87 days	*C. parvum*	Norovirus	Work as a nurse, contact with recreative water, treated with phenoxymethylpenicillin	Peri-urban

M: male, F: female, y.o: years old, IL2r: interleukin 2 receptor, CNI: calcineurin inhibitor, MMF: mycophenolic mofetil

Table III. Clinical description and epidemiological characteristics of patients with microsporidiosis (N=4)

Tableau III. Description clinique et caractéristiques épidémiologiques des patients avec une microsporidiose (N=4)

Sex, Age number of graft	**Season of infection**	**Time since transplantation**	**Induction and maintenance immuno-suppressive therapies**	**Symptoms**	**Time to diagnosis**	**Species**	**Co-infection**	**Environmental risk factor**	**Living place**
F, 55 y.o 3rd graft	Autumn	73 months	Depleting therapy CNI + MMF	Diarrhea, dehydration, fever, abdominal pain, asthenia	39 days	*E. bieneusi*	No	Contact with animals	Urban
F, 58 y.o 1st graft	Summer	24 months	Anti-IL2r CNI + MMF	Abdominal pain, diarrhea, vomiting	40 days	*E. bieneusi*	No	Unwashed vegetables	Rural
M, 75 y.o 1st graft	Spring	13 months	Anti-IL2r CNI + MMF	Asthenia, abdominal pain, diarrhea, vomiting, weight loss (10 kg)	29 days	*E. bieneusi*	No	None	Urban

F, 58 y.o 1st graft	Summer	2 months	Anti-IL2r CNI + MMF	Fever, asthenia, abdominal pain, diarrhea, vomiting, dehydration, weight loss (0.5 kg)	14 days	*E. bieneusi*	No	Contact with his cat, previous antibiotic therapy with quinolone	Rural

M: male, F: female, y.o: years old, IL2r: interleukin 2 receptor, CNI: calcineurin inhibitor, MMF: mycophenolic mofetil