

## Prevalence, clinical presentation and treatment outcome of cryptosporidiosis in immunocompetent adult patients presenting with acute diarrhoea

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### Abstract

**Objective:** To document the prevalence, clinical presentation and treatment outcome of cryptosporidiosis in immunocompetent adult patients presenting with acute diarrhoea.

**Methods:** This prospective cohort study was conducted at the Sindh Institute of Urology and Transplantation Karachi from February 1, till September 30, 2012. All immunocompetent adult patients who presented with acute diarrhoea to the gastroenterology clinic at SIUT were included. Data collection sheet was filled and stool studies sent. Modified acid fast stain of stool was performed for cryptosporidium. SPSS 20 was used for statistical analysis.

**Results:** There were 105 patients with acute diarrhoea. Fifty three (50.4%) were males. The mean age was 34±8.4 years. Of 105, 58 (55%) patients had cryptosporidium isolated in stool studies. Patients with cryptosporidiosis had statistically significant greater stool frequency per day ( $p<0.001$ , OR=12.7; CI [4.4-37.1]), abdominal pain ( $p<0.001$ , OR= 19.8 [6.1-64.1]), vomiting ( $p<0.001$ , OR=7.3 [2.7-19.9]), low grade fever ( $p<0.001$ , OR=8.5 [3.5-20.8]), fatigue ( $p<0.001$ , OR=8.4 [3.2-21.6]) and dehydration and a shorter duration of illness with more watery diarrhoea. All 58 patients reported resolution of diarrhoea after 7 days of treatment with nitazoxanide. However, 40 (70.1%) patients reported recurrence of diarrhoea within 6 weeks of treatment.

**Conclusion:** Our study demonstrates a high prevalence of cryptosporidiosis in immunocompetent adult patients. Nitazoxanide is the recommended antimicrobial drug for cryptosporidiosis.

**Keywords:** Diarrhoea, Cryptosporidiosis, Immunocompetent, Adult, Nitazoxanide. (JPMA 64: 613; 2014)

### Introduction

Human cryptosporidiosis is caused by infection with the Apicomplexa protozoan of the genus *Cryptosporidium*. Among the more common species are *Cryptosporidium hominis*, for which humans are the only natural hosts, and *Cryptosporidium parvum*, which infects bovines as well as humans.<sup>1,2</sup> Its infectious forms are oocysts which are excreted in the host's faeces. The principal zoonotic reservoirs are humans, cattle and other domestic animals. It is transmitted by the faeco-oral route, person-to-person or from animal to person, as well as by ingestion of contaminated water or food.<sup>3</sup>

The frequency of cryptosporidiosis has not been well-defined. About 30% of the adult population of the United States are seropositive with over 10,500 cases reported in 2008.<sup>4</sup> In persons with AIDS from developing countries, the rates of cryptosporidiosis are higher ranging from 12 to 48%.<sup>1</sup>

The main clinical presentation in immunocompetent patients is watery diarrhoea of variable severity, abdominal pain, low grade fever, nausea, vomiting and

dehydration.<sup>2,5</sup> Cryptosporidiosis usually causes self limited diarrhoea in otherwise healthy adult patients but can cause prolonged and recurrent diarrhoea in children. Patients presenting with these symptoms at our institution are often prescribed ciprofloxacin and/or metronidazole empirically. This appears to be a common practice by general physicians throughout the world.<sup>6,7</sup>

In Pakistan, there is a low level of awareness regarding cryptosporidiosis in the immunocompetent adult population and this is true worldwide, with only a few studies published to date.<sup>8,9</sup>

The objectives of this study are to document the prevalence, clinical presentation and treatment outcome of cryptosporidiosis in immunocompetent patients presenting with acute diarrhoea to the outpatient gastroenterology clinic at our institution. Most importantly, the purpose of our study is to raise awareness regarding cryptosporidiosis as a diarrhoeal pathogen in Pakistan.

### Materials and Methods

After obtaining an approval by the Ethical Review Board of Sindh Institute of Urology and Transplantation (SIUT), this prospective cohort study was conducted at SIUT in Karachi between 1st of February and 30th of September in 2012. A total of 105 immunocompetent adult patients

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above the age of 16 years that presented within the study period with acute diarrhoea to the gastroenterology clinic at SIUT were included after taking written informed consent. Patients were excluded if they had a prior known diagnosis of cryptosporidiosis, or any history of immunodeficiency such as HIV infection, haematological malignancy, solid organ transplantation or receiving steroids or other immunosuppressive drugs. Patients were also excluded if they had taken nitazoxanide, paramomycin or azithromycin within the last 4 weeks prior to presentation.

Acute diarrhoea was defined as  $\geq 3$  episodes of loose stools per day with or without abdominal pain, vomiting and/or fever. Empirical treatment was prescribed by the gastroenterologists at their own discretion and not as part of the study protocol. After completion of the clinic visit with the gastroenterologist, every patient with acute diarrhoea who met the inclusion criteria was then recruited into our study on the same visit. The data collection sheet was filled with documentation of demographics and clinical presentation which included the presence of fever, abdominal pain, duration of diarrhoea and description of stools regarding consistency, frequency, volume and presence of blood and/or mucus. Stool studies were requested which included faecal leukocytes, culture and sensitivity and 3 samples for ova and parasites, including cryptosporidium. Patients were given a follow-up appointment in 1 week to review the stool reports and clinical condition.

At the follow-up visit, which was the 2nd visit, patients who were reported to have stool pathogens other than cryptosporidium were treated as per standard guidelines. If stool was found to be positive for cryptosporidium, we prescribed nitazoxanide 500mg twice daily for 7 days. Although 3 days of therapy are recommended in immunocompetent adults, we chose a longer treatment period based on our clinical experience, which seemed to suggest an unsatisfactory response to shorter therapy. In case of co-infection of Cryptosporidia with Giardia and/or Entamoeba Histolytica, our study protocol did not recommend any additional antiprotozoal other than nitazoxanide. Antimotility agent loperamide 4 mg stat dose, then 1 mg after each stool to a maximum of 16mg per day was recommended in our protocol for only those patients with more than 4 stools per day and who did not have bacillary dysentery. If co-infection of cryptosporidia with Salmonella and/or Shigella was found, an antimicrobial as per culture and sensitivity was warranted only if there were  $>6$  stools per day, fever with nausea, vomiting, abdominal pain and/or bloody stools.

A follow-up visit at 2 weeks after prescribing nitazoxanide

(3rd visit) was scheduled for patients with cryptosporidiosis to assess clinical response to 1 week of nitazoxanide treatment. Patients were called by telephone if they did not show up for this visit and their response was documented by phone.

Finally, contact was made by telephone at 6 weeks since prescribing nitazoxanide to enquire whether diarrhoea was ongoing, resolved or had relapsed during the past 6 weeks.

Data were entered in SPSS version 20 software for analysis. Continuous variables were employed for mean age and standard deviation. Categorical variables were analyzed as frequencies and percentages. Univariate analysis was done for clinical presentation and treatment outcomes.

### Microbiology

Patients were asked to submit 3 consecutive stool samples which were collected in plastic containers. The specimens were processed immediately for enteropathogenic bacteria, Cryptosporidium, as well as other protozoan and nematode parasites. Each specimen was examined by the naked eye for consistency, colour, and the presence of worms, mucous, or blood. Wet mount preparations of the specimens in physiologic saline and iodine were examined for protozoan cysts and ova.

For the identification of Cryptosporidium, all stool specimens were concentrated using the formalin-ethyl acetate method by centrifugation at 8003g. Smear was made from the sediment and fixed by passing over a flame and stained by modified Ziehl Neelsen method, decolorized by 1% sulphuric acid, then washed with water and counterstained by methylene blue, then air dried and observed under high power and oil immersion lens. Three smears were made for each specimen. Stool specimens were considered positive if oocysts between 4 mm and 6 mm in diameter that stained bright pink, usually within a clear halo, and with typical morphology, were seen. Specimens were further screened for Escherichia coli, Salmonella spp., and Shigella spp. by culturing on MacConkey agar and Salmonella Shigella agar.

### Results

A total of 105 patients presented to the gastroenterology OPD with acute diarrhoea during the 8 months study period. Of 105, 58 (55%) patients had cryptosporidium isolated in stool studies in the very first sample.

The demographic details of the study population is shown in Table-1.

There were 53 (50.4%) males and 52 (49.5%) females. The

mean age was 34±8.6 years. There was no statistical significance for prevalence of cryptosporidiosis in relation to age, sex, residence and prior admission within the past 12 weeks. However, statistical significance for cryptosporidia infection was found for patients who had a diagnosis of coeliac disease or sprue.

The clinical presentation of the patients is shown in Table-2.

Patients diagnosed with cryptosporidiosis had statistically significant greater stool frequency per day, abdominal pain, vomiting, low grade fever, fatigue and dehydration as compared with those that did not have

**Table-1:** Demographic Data of 105 Patients Presenting with Acute Diarrhoea to the Outpatient Gastroenterology Clinic.

Demographics		Cryptosporidium Present in Stool n=58 (55%)	Cryptosporidium Absent in Stool n=47 (45%)	Standard Deviation	p-value
Gender	Male	31 (53.4%)	22 (46.8%)	34.01 ± 8.6	
	Female	27 (46.6%)	25 (53.2%)		
Age in Years	< 20	5 (8.6%)			
	21 - 30	20 (34.5%)	17 (36.2%)		
	31 - 40	23 (39.7%)	21 (44.7%)		
	41 - 50	7 (12.1%)	8 (17%)		
	>50	3 (5.2%)	1 (2.1%)		
Residence of Patients	Rural	4 (6.9%)	2 (4.3%)		
	Urban	54 (93.1%)	45 (95.7%)		
Health Care Worker	Non-HCW	55 (94.8%)	45 (95.7%)		
	HCW	3 (5.2%)	2 (4.3%)		
Co-morbidities	No co-morbidities	22 (37.9%)	10 (21.3%)		
	Co-morbidities:	36 (62.1%)	37 (78.7%)		
	Viral hepatitis	22	16		
	CRF	5	2		
	Diabetes	3	4		
Admission in hospital during the past 12 weeks	Celiac disease, sprue	4	14		
	Other diagnosis	2	1		
	No	48 (82.8%)	28 (59.5%)		
	Yes	10 (17.2%)	19 (40.5%)		

HCW: Health Care Worker. CRF: Chronic Renal Failure.

**Table-2:** Clinical presentation of patients with acute diarrhoea.

Clinical parameters		With Cryptosporidiosis n=58 (55 %)	Without Cryptosporidiosis n=47 (45 %)	Standard Deviation	P-value	OR (95% C.I)
Duration of Illness(days)	<5	43 (74.1%)	13 (27.7%)	6.3 ± 3.13	<0.001	0.1 (0.05-0.31)
	≥ 5	15 (25.9%)	34(72.3%)			
Frequency of Diarrhoea	Single Episode	8 (13.8%)	3 (6.4%)	5.4 ± 3.20	<0.001	12.7 (4.4 - 37.1)
	Recurrent Episodes	50 (82.2%)	44 (93.6%)			
Stool Frequency per day	< 5	23 (39.7%)	42 (89.4%)			
	≥ 5	35 (60.3%)	5 (10.6%)			
Stool Consistency	Watery	50 (86.2%)	9 (19.1%)		<0.001	0.03 (0.01 - 0.10)
	Formed	8 (13.8%)	38 (80.9%)			
Blood in Stool	Yes	3 (5.2%)	9 (19.1%)		0.02	0.23 (0.58 - 0.9)
Mucus in Stool	Yes	8 (13.8%)	22 (46.8%)		<0.001	0.1 (0.07 - 0.46)
Abdominal Pain	Yes	54 (74%)	19 (26%)		<0.001	19.8 (6.1 - 64.1)
Vomiting	Yes	30 (83.3%)	6 (16.7%)		<0.001	7.3 (2.7 - 19.9)
Weight Loss	Yes	17 (41.5%)	24 (58.5%)		0.02	0.3 (0.1 - 0.8)
Fever during Diarrhoea	Yes	42 (79.2%)	11 (20.8%)		<0.001	8.5 (3.5 - 20.8)
Dehydration	Yes	50 (71.4%)	20 (28.6%)		<0.001	8.4 (3.2 - 21.6)
Headache	Yes	19 (57.6%)	14 (42.4%)		0.74	
Fatigue	Yes	56 (57.7%)	41 (42.3%)		0.07	4.0 (0.78 - 21.3)
Joint Pain	Yes	25 (49%)	26 (51%)		0.21	

**Table-3:** Pathogens isolated in stool studies of 105 patients with acute diarrhoea.

Pathogens	Number	Frequency
No Pathogen	24	22.9%
Cryptosporidium	43	41.0%
Cryptosporidium with other pathogens	15	14.3%
EntamoebaHistolytica	4	3.8%
Giardia	3	2.9%
Salmonella	12	11.4%
Shigella	2	1.9%
Salmonella / Shigella	2	1.9%
Total	105	100.0%

cryptosporidiosis. The duration of illness was shorter in patients with cryptosporidiosis. They also had more watery diarrhoea and were less likely to have blood and mucous in stools as compared with those that did not have cryptosporidiosis.

Of 105 patients stool samples, pathogens were isolated in 81 patients (77%). Of 105 with acute diarrhoea, fifty eight (55%) patients had Cryptosporidium of which 43 (40.9 %) had Cryptosporidium alone whereas 15 (14.2 %) were co-infected with one or more other species. Of those co-infected, the other pathogens included Entamoeba Histolytica in 4 (3.8%), Giardia in 3 (2.8%), Isospora Belli in 3 (2.8%), Salmonella and/or Shigella in 3 (2.8%) and Ascaris Lumbricoides in 2 (1.90%). Pathogens other than cryptosporidium isolated in stool studies are mentioned in Table-3.

Empirical antibiotics were prescribed by gastroenterologists in 68 (64.7%) patients at their first visit. Ciprofloxacin 500mg twice daily and metronidazole 400mg three times daily in combination was most commonly prescribed in 60 of 105 (57%) patients; 7 (6.6%) patients got ciprofloxacin alone and 1 (.95%) got metronidazole alone. Empirical ciprofloxacin and/or metronidazole at the first visit was prescribed in 31 of 58 (53.4%) patients with cryptosporidiosis isolated in stool, either alone or co-infected, and in 18 of 43 (41.8%) patients with cryptosporidiosis alone.

Of 58 patients who were diagnosed with cryptosporidiosis and treated with 1 week of nitazoxanide, all 58 (100%) reported resolution of diarrhoea at 2 weeks. However, at 6 weeks follow-up by phone, only 17 (29.9%) reported having been free of diarrhoea whereas 40 (70.1%) patients reported recurrence of diarrhoea within the 6 week period. One patient could not be contacted at 6 weeks.

## Discussion

Our study has shown a high frequency of

cryptosporidiosis in immunocompetent adult patients presenting with acute diarrhoea, with more than half found to have this protozoal illness. Studies worldwide have shown a prevalence of upto 47% cryptosporidiosis in immunocompetent patients.<sup>10</sup> Studies from Pakistan have reported 3.9% prevalence in chronic diarrhoea patients<sup>11</sup> and 40% prevalence in immunosuppressed patients.<sup>12</sup> Another study from Karachi revealed a prevalence of 35.6% in stool samples collected from the general population.<sup>13</sup> To the best of our knowledge, this is the first prospective study of cryptosporidiosis in immunocompetent adults presenting with acute diarrhoea in Pakistan.

Physicians should consider cryptosporidiosis in patients presenting with a short history of acute, watery diarrhoea with frequency of greater than 5-6 stools per day. A stool test for modified acid fast stain for Cryptosporidium must be requested. This is a cheap and simple method for diagnosis and should be performed by microbiologists as part of their routine stool studies.<sup>14</sup>

Misuse of antibiotics in diarrhoea patients is worldwide.<sup>7</sup> Out of 58 patients with cryptosporidiosis, over half received either ciprofloxacin and/or metronidazole empirically. Although two-thirds of our patients were empirically prescribed ciprofloxacin, only 19 patients had Salmonella/Shigella subsequently isolated in stool studies. Ciprofloxacin can prolong the carrier state in case of Salmonella bacillary dysentery.<sup>15</sup> Guidelines recommend that antibiotics should not be given in uncomplicated salmonella gastroenteritis and only prescribed if patients present with fever, bloody diarrhoea, abdominal tenderness, nausea and vomiting and >6 stools per day.<sup>15</sup> Increasing resistance has been reported to quinolones with Salmonella, Shigella and Campylobacter.<sup>16</sup> Moreover, although metronidazole at a dose of 400mg every 8 hours is appropriate for giardiasis, in case of amoebic dysentery, a much higher dose of 750mg every 8 hours together with diloxanidefuroate is recommended. Therefore, empirical antimicrobials need to be given appropriately only after careful evaluation of a patient with diarrhoea, rather than as a standard protocol.<sup>15</sup>

FDA has approved nitazoxanide for treatment of cryptosporidiosis and giardiasis. Nitazoxanide is a nitrothiazolyl-salicylamide derivative which has shown activity against cryptosporidium and is effective in its treatment.<sup>17</sup> Other enteric protozoan infections caused by Giardia intestinalis, Entamoebahistolytica, Blastocystishominis and Isospora belli can also be treated with nitazoxanide.<sup>18</sup> Some studies have shown efficacy of

nitazoxanide against *Clostridium difficile* colitis.<sup>19</sup> Nitazoxanide has also been used for severe rotavirus diarrhoea.<sup>20</sup> The side effects of nitazoxanide include nausea, vomiting, epigastric pain and headache.

Guidelines recommend a 3-day course of nitazoxanide, 500mg twice daily in adults.<sup>21</sup> Although we gave 7 days of therapy and a satisfactory treatment response was obtained in the short term, there was a high recurrence rate.<sup>21</sup> Paromomycin and/or azithromycin in combination with nitazoxanide have been tested in double blind randomized trials for the treatment of cryptosporidiosis in immunocompromised patients such as those with HIV/AIDS, and the results have been encouraging.<sup>18,22,23</sup> Given the high recurrence rate, further studies need to be conducted regarding optimal dosing, duration and antimicrobial combinations for the treatment of cryptosporidiosis in immunocompetent adult patients.

Currently nitazoxanide is not recommended for empirical use in acute diarrhoea; however there are some double blind placebo controlled trials being conducted and their results are statistically significant.<sup>24</sup> Our study suggests that we could consider prescribing nitazoxanide empirically, rather than ciprofloxacin/metronidazole, in immunocompetent adults that present with watery, non-bloody diarrhoea.

A significant number of our patients with cryptosporidiosis had been diagnosed with coeliac disease or tropical sprue. Several studies have shown that cryptosporidiosis itself causes blunting of intestinal villi.<sup>8</sup> It is conceivable that in those study patients that had a diagnosis of tropical sprue based on the finding of villous blunting alone, the diagnosis was actually cryptosporidiosis.

Cryptosporidiosis is a waterborne disease, most commonly transmitted from person to person, via contaminated water and food. *Cryptosporidium* is usually chlorine resistant and not killed by chlorination.<sup>25</sup> Boiling of water is the most reliable means of killing this organism.<sup>5</sup> Filtration with pore size of 1 µm or less is also an effective method.<sup>26</sup> Other preventive measures include practicing good hygiene, not swimming when ill with diarrhoea, washing hands appropriately and avoiding contaminated water.<sup>27</sup>

## Conclusion

Our study has demonstrated a high prevalence of cryptosporidiosis in immunocompetent adult patients. Cryptosporidiosis is an emerging infectious disease in Pakistan. Physicians need to consider cryptosporidiosis

in immunocompetent patients presenting with acute, watery diarrhoea and request a modified acid fast stain for cryptosporidium. Nitazoxanide is the recommended antimicrobial drug for cryptosporidiosis. However the recurrence rate of diarrhoea is high. Further randomized controlled studies may be needed regarding optimal management of cryptosporidiosis.

## References

- Ramirez NE, Ward LA, Sreevatsan S. A review of the biology and epidemiology of cryptosporidiosis in humans and animals. *Microbes Infect* 2004; 6: 773-85.
- Hunter PR, Hughes S, Woodhouse S, Raj N, Syed Q, Chalmers RM, et al. Health sequelae of human cryptosporidiosis in immunocompetent patients. *Clin Infect Dis* 2004; 39: 504-10.
- Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhoea. *Gastroenterology* 2009; 136: 1874-86.
- Yoder JS, Herral C, Beach MJ. Cryptosporidiosis surveillance — United States, 2006-2008. *MMWR Surveill Summ* 2010; 59: 1-14.
- Yoder JS, Wallace RM, Collier SA, Beach MJ, Hlavsa MC. Cryptosporidiosis surveillance—United States, 2009-2010. *MMWR Surveill Summ* 2012; 61: 1-12.
- Nasir MA, Hashmi RI, Ahmad NS. Drug utilization patterns in Rawalpindi and Islamabad, Pakistan. *J Pak Med Assoc* 2012; 62: 426-9.
- Diniz-Santos DR, Silva LR, Silva N. Antibiotics for the empirical treatment of acute infectious diarrhoea in children. *Braz J Infect Dis* 2006; 10: 217-27.
- Leav BA, Mackay M, Ward HD. *Cryptosporidium* species: new insights and old challenges. *Clin Infect Dis* 2003; 36: 903-8.
- Lee JK, Song HJ, Yu JR. Prevalence of diarrhoea caused by *Cryptosporidium parvum* in non-HIV patients in Jeollanam-do, Korea. *Korean J Parasitol* 2005; 43: 111-4.
- Youssef FG, Adib I, Riddle MS, Schlett CD. A review of cryptosporidiosis in Egypt. *J Egypt Soc Parasitol* 2008; 38: 9-28.
- Yakoob J, Abbas Z, Beg MA, Naz S, Khan R, Islam M, et al. Prevalences of *Giardia lamblia* and *Cryptosporidium parvum* infection in adults presenting with chronic diarrhoea. *Ann Trop Med Parasitol* 2010; 104: 505-10.
- Baqai R, Anwar S, Kazmi SU. Detection of cryptosporidium in immunosuppressed patients. *J Ayub Med Coll Abbottabad* 2005; 17: 38-40.
- Baqai R, Hafiz S, Zuberi S. Cryptosporidiosis in Karachi. *J Pak Med Assoc* 1992; 42: 174.
- Shoail S, Tauheed S, Hafiz A. Frequency of cryptosporidium in childhood diarrhoea—importance of modified acid fast technique. *J Ayub Med Coll Abbottabad* 2003; 15: 3-5.
- Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, et al. Practice guidelines for the management of infectious diarrhoea. *Clin Infect Dis* 2001; 32: 331-51.
- Zafar A. *Campylobacter* and emerging antimicrobial resistance. *J Pak Med Assoc* 2004; 54: 343-4.
- Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhoea caused by *Cryptosporidium parvum*: a prospective randomized, double-blind, placebo-controlled study of Nitazoxanide. *J Infect Dis* 2001; 184: 103-6.
- Rossignol JF. *Cryptosporidium* and *Giardia*: treatment options and prospects for new drugs. *Exp Parasitol* 2010; 124: 45-53.
- Musher DM, Logan N, Bressler AM, Johnson DP, Rossignol JF. Nitazoxanide versus vancomycin in *Clostridium difficile* infection: a randomized, double-blind study. *Clin Infect Dis* 2009; 48: e41-6.

20. Rossignol JF, El-Gohary YM. Nitazoxanide in the treatment of viral gastroenteritis: a randomized double-blind placebo-controlled clinical trial. *Aliment Pharmacol Ther* 2006; 24: 1423-30.
  21. Pantenburg B, Cabada MM, White AC Jr. Treatment of cryptosporidiosis. *Expert Rev Anti Infect Ther* 2009; 7: 385-91.
  22. Thielman NM, Guerrant RL. Clinical practice. Acute infectious diarrhoea. *N Engl J Med* 2004; 350: 38-47.
  23. Allam AF, Shehab AY. Efficacy of azithromycin, praziquantel and mirazid in treatment of cryptosporidiosis in school children. *J Egypt Soc Parasitol* 2002; 32: 969-78.
  24. Rossignol JF, Lopez-Chegne N, Julcamoro LM, Carrion ME, Bardin MC. Nitazoxanide for the empiric treatment of pediatric infectious diarrhoea. *Trans R Soc Trop Med Hyg* 2012; 106: 167-73.
  25. Kaplan JE, Benson C, Holmes KH, Brooks JT, Pau A, Masur H. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009; 58: 1-207.
  26. Addiss DG, Pond RS, Remshak M, Juranek DD, Stokes S, Davis JP. Reduction of risk of watery diarrhoea with point-of-use water filters during a massive outbreak of waterborne *Cryptosporidium* infection in Milwaukee, Wisconsin, 1993. *Am J Trop Med Hyg* 1996; 54: 549-53.
  27. Shirley DA, Moonah SN, Kotloff KL. Burden of disease from cryptosporidiosis. *Curr Opin Infect Dis* 2012; 25: 555-63.
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