Cryptosporidiosis and Other Enteric Protozoan Infections in AIDS-related Diarrhea in Thailand

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Diarrhea in AIDS

- **A substantial number of HIV- infected patients suffer from diarrhea caused by a wide range of opportunistic and non-opportunistic pathogens.**
- **It is associated with significant morbidity and mortality.**
- **The accurate identification on the causes of diarrhea allows appropriate treatment.**

Cryptosporidiosis

- **Human infection is caused by at least 8 *Cryptosporidium species/genotypes.***
- ***C. parvum* and *C. hominis* are the most common species infect human.**
- ***C. meleagridis, C. felis, C. canis*, *C. suis*, *C. muris*, and *Cryptosporidium* cervine genotype also infect human.**

Cryptosporidiosis

- **In immonocompetent person**
  - **Asymptomatic carriage**
  - **Acute, self -limited diarrhea**
  - **Persistent diarrhea that may continue for weeks**
- **Supportive care with IV or oral rehydration fluid to correct the dehydration**
- **No specific Treatment is required**

Cryptosporidiosis

- **Clinical manifestations in patients with AIDS**
  - **Asymptomatic infection**
  - **Transient infection : CD4 > 200/ cu.mm**
  - **Chronic diarrhea ≥ 2 months, persistent oocyst when CD4 < 100/cu.mm.**
  - **Fulminant disease, > 2 L of watery diarrhea/ daily if CD4 < 50/ cu.mm.**
- **Shorter survival than those without cryptosporidiosis.**

Treatment of Cryptosporidiosis

- Antimicrobial therapy
- Immunotherapy
- Symptomatic anti-diarrheal treatment

Immune Reconstitution

- **Immune reconstitution using HAART**
- **Useful as a treatment and secondary prophylaxis for cryptosporidiosis in HIV-infected patients.**
- **Protease inhibitors reduce *C. parvum* sporozoite host cell invasion and inhibit parasite development in vitro.**
- **The inhibitory effect was increased when paromomycin was combined with the PIs.**

Passive Immunotherapy

- **Oral bovine serum concentrate improved symptoms and reduced oocyst shedding in experimental cryptosporidial diarrhea**
- **Colostrum from cows hyperimmunized with *C. parvum* oocysts achieved limited success in both human and non-human hosts.**

Antimicrobial Therapy

- **Macrolide**
  - Oral spiramycin
  - Azithromycin
  - Clarithromycin
- **Aminoglycoside**
  - Paramomycin
- **Nitazoxanide**

Nitazoxanide

- **2-(acetyloxy)-N-(5-nitro-2-thiazolyl) benzamide, a synthetic oral antiparasitic agent, is effective against broad range of protozoa and helminths including *Cryptosporidium spp*.**
- **It has been licensed for the treatment of giardia-associated diarrhea and cryptosporidial diarrhea in non-HIV infected children since December 2002.**
- **It is the first and only US FDA-approved drug for cryptosporidiosis so far.**

Nitazoxanide

- In 50 HIV-negative children with cryptosporidiosis
  - **Diarrheal resolved in 56% in nitazoxanide treated group compared to 23% in placebo group (P=0.037).**
  - **Oocyst eradication in 52%in nitazoxanide treated group compared to 14% in placebo group (P=0.007).**
  - **Mortality 18% at day 8th in placebo group compared to 0% in nitazoxanide treated group (P=0.04)**
- **No benefit was shown in 50 HIV+ve children, diarrhea resolved after a 2nd course of treatment, but few of them had parasitological clearance.**

Amadi et al. Lancet 2002; 360:1375- 80

Nitaxozanide

- **A phaseII placebo- controlled study of nitazoxanide in AIDS subjects in Mexico**
  - **a 14-day course of nitazoxanide at a dose of 1,000 mg bid was effective in treating cryptosporidiosis in patients with CD4 s > 50**

    (parasitological response 67% vs 25% in placebo group)

Nitazoxanide

- **A phaseII placebo- controlled study of nitazoxanide in AIDS subjects in Mexico**
  - **–In patients with CD4 <50 cu.mm, the response rate of nitazoxanide therapy was not significant difference from placebo.**
  - **– Higher doses and /or longer duration of therapy may be needed to obtain responses in these severely immunocompromised patients**

OBJECTIVES

- To determine the prevalence of protozoan pathogens associated with diarrhea in HIV-infected patients in Thailand.
- To compare clinical manifestation of diarrhea caused by these pathogens.
- To determine the efficacy and safety of nitazoxanide treatment for cryptosporidiosis.

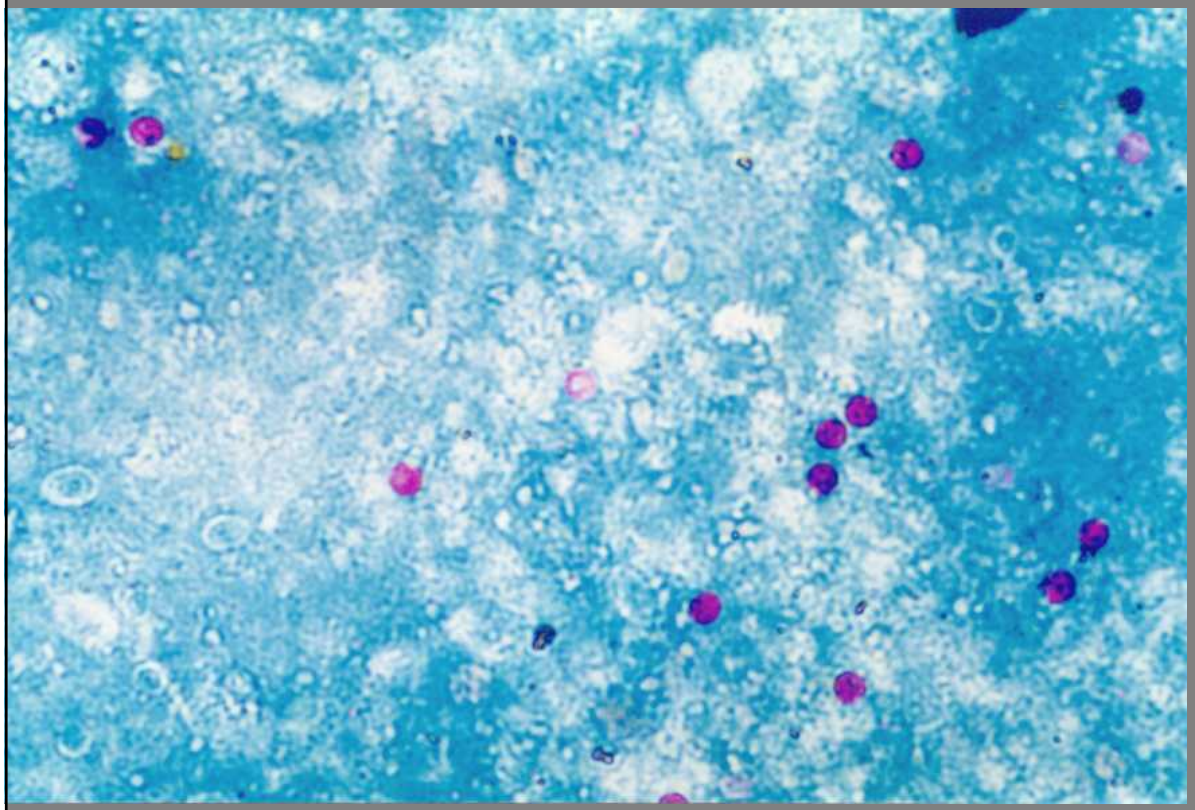
Material And Methods

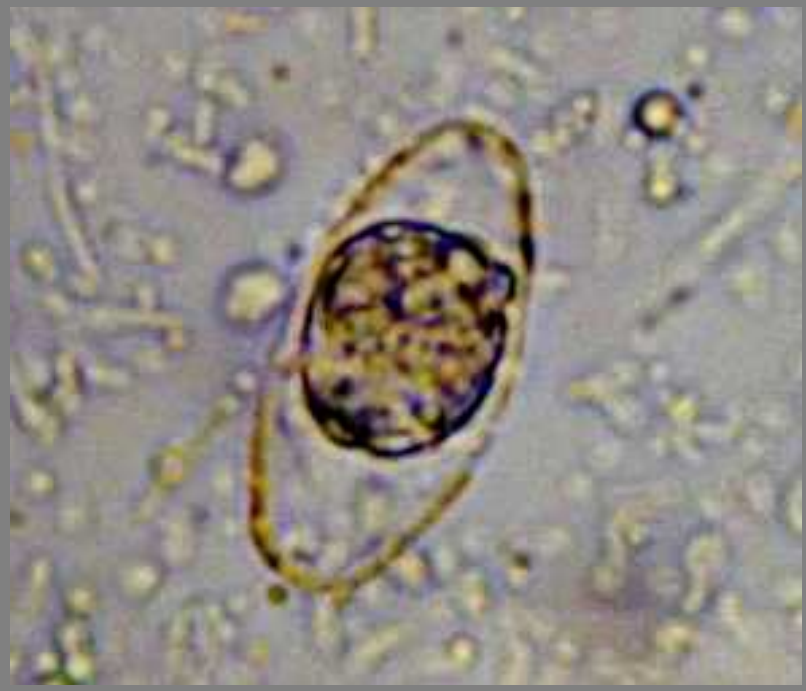
- A laboratory-based coprodiagnostic investigation conducted at Siriraj Hospital and Bamrasnaradura Institue, Bangkok, Thailand

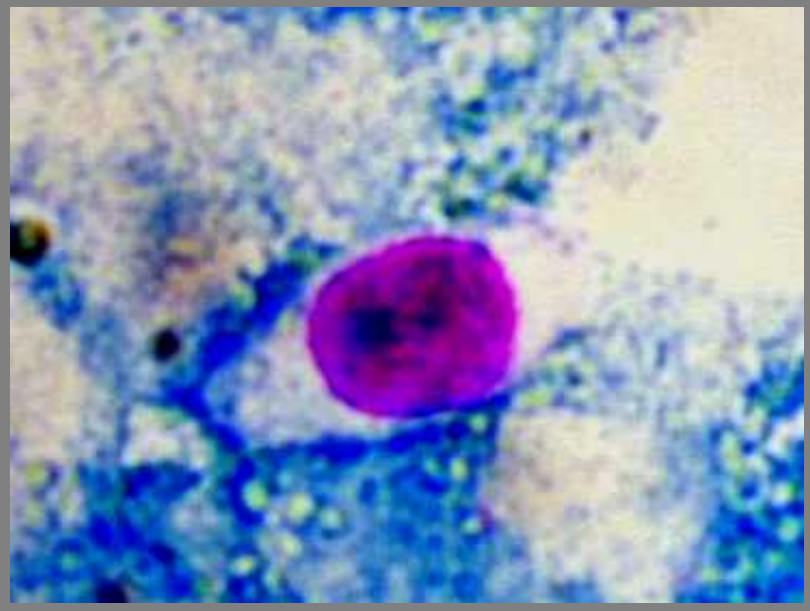
- All patients with diarrhea were asked to produce a stool sample for
  - simple wet smear and formalin-ether concentrates
  - modified Ziehl-Neelsen stained smear
  - enteric bacterial and mycobacteria culture
  - *Clostridium difficile* toxin A assay
  - Modified trichrome blue stained smear

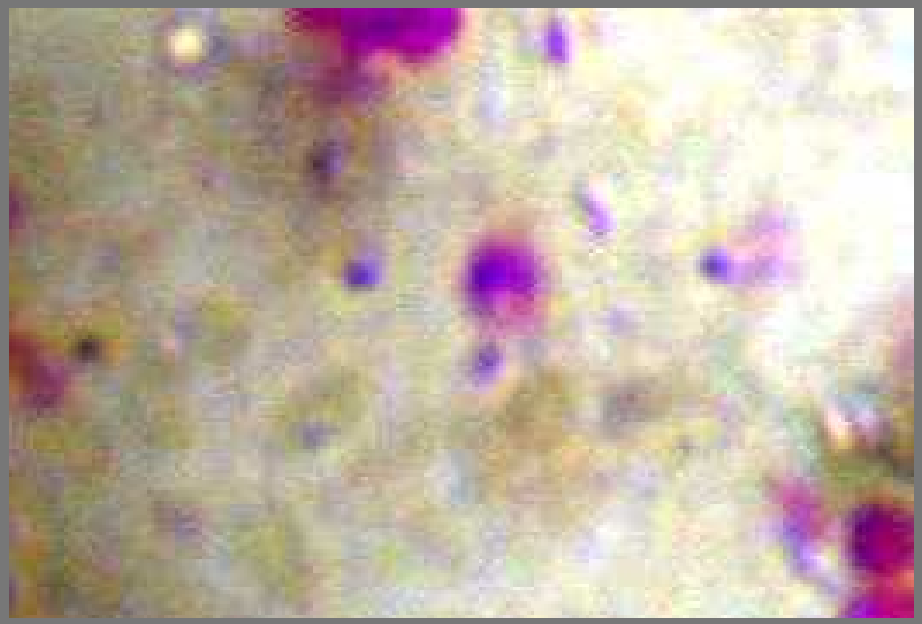
Material And Methods

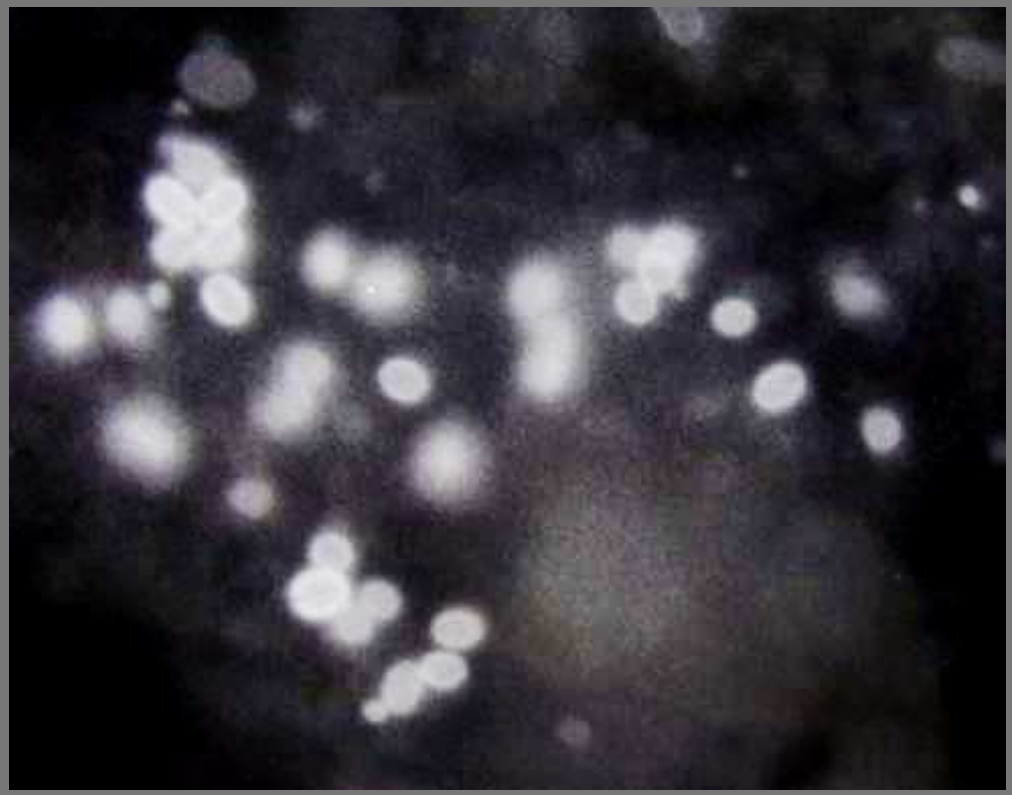
- The identification of Cryptosporidium oocyst was confirmed by Meriflour Crytosporidium-Giardia monoclonal direct immunofluorescence detection kit.
- The identification of Microsporidia was confirmed and speciated by thin sectioning electron microscopy.

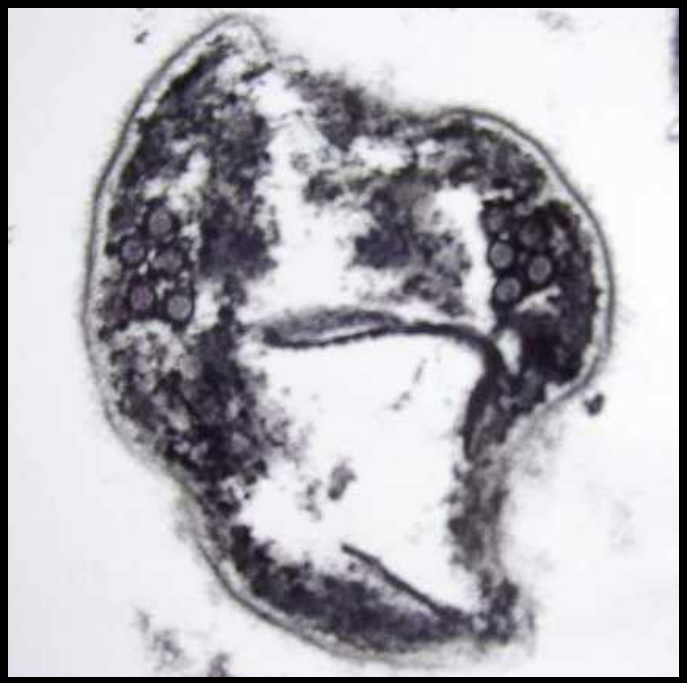


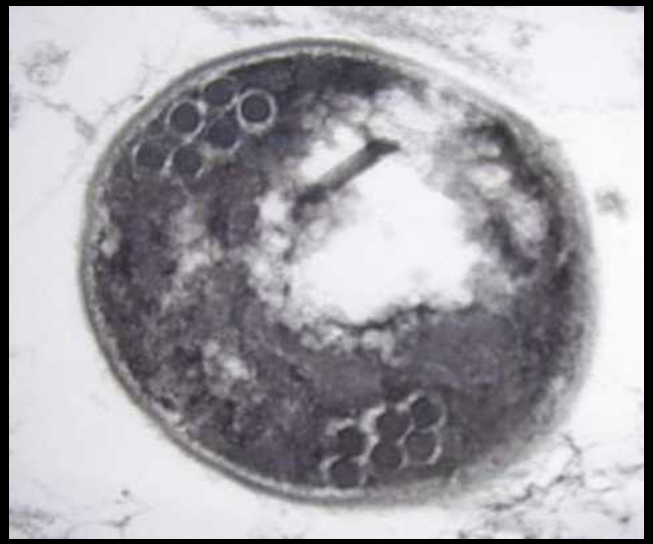












A Randomized Placebo – Controlled Trial of Nitazoxanide for the Treatment of Cryptosporidiosis

Material and Methods

- **Inclusion Criteria**
  - **HIV infection and CD4 counts ≤ 50 /cu.mm.**
  - **Age > 13 years**
  - **Presence of oocyst of *C. parvum*.**
  - **At least 3 bowel movements per day, on average, during the 5 days prior to enrollment based upon the observation of the hospital staff, and on at least 5 days a week, on average, for 21 days prior to enrollment.**
  - **Willingness to remain hospitalized for 5 days prior to enrollment and for the first 14 days of the study.**
  - **In the case of females, adequate birth control.**

Material and Methods: exclusion criteria

- Inability to tolerate oral medications.

- Life expectancy < 120 days in the opinion of the investigator

- Active CMV colitis, *C. difficile* colitis, amebiasis, giardiasis, salmonellosis, shigellosis, campylobacteriosis, inflammatory bowel disease, diarrhea secondary to another documented pathogen (other than microsporidia) or symptomatic MAC disease.

-Need for continuing use of any medications with potential anticryptosporidial activity including paromomycin, azithromycin, clarithromycin, spiramycin, bovine colostrum, monoclonsl anticryptospiridial antibody preparations, etc.

Material and Methods

- **Study Procedure**
  - **Nitazoxanide/ placebo 1,000 mg bid for 4 weeks. follow by 1,500 mg bid for 4 weeks**
  - **Patients were examined every 2- week during the 8 week treatment and at 2, 4, 6 week after Rx**
  - **A stool sample was collected at each visit**
  - **Two stool samples were collected at 8-week of treatment and 6-week of follow up**

Material and Methods

- **Study Procedure**
  - **–Treatment were discontinued in the event of parasitological “cure” and “ well” clinical response on two consecutive visits.**
  - **– Patients with persistent shedding of *Cryptosporidium* oocyst received nitazoxanide open label treatment of the same dosage regimen.**

Outcome

- **Clinical response**
  - **Well : < 3 bowel movements/d over the last 5 days**
  - **Continuing illness**
  - **Clinical relapse/ reinfection**
- **Parasitological response**
  - **Eradication: no cryptosporidium oocyst in 2 stool samples collected at the end of treatment**
  - **Persistence**
  - **Relapse or reinfection**

Outcome

- **Therapeutic response**
  - **Cure ; well + eradication**
  - **Failure; Continuing illness or persistence**
  - **Relapse; a change in therapeutic response from cure at the end of treatment to failure at the follow up examination.**

RESULTS

- 1,138 fecal samples from 909 patients were screened between November 1999- July 2004
  - 683 (75.2) patients with diarrhea
    - 219 (24.1%) patients with diarrhea for <1 week.
    - 109 (12%) patients with diarrhea between one to less than 4 weeks.
    - 355 (39.1%) patients with diarrhea for ≥4 weeks.
  - 54(5.9) patients without diarrhea
  - No data available in 172 (18.9%) patients.

RESULTS

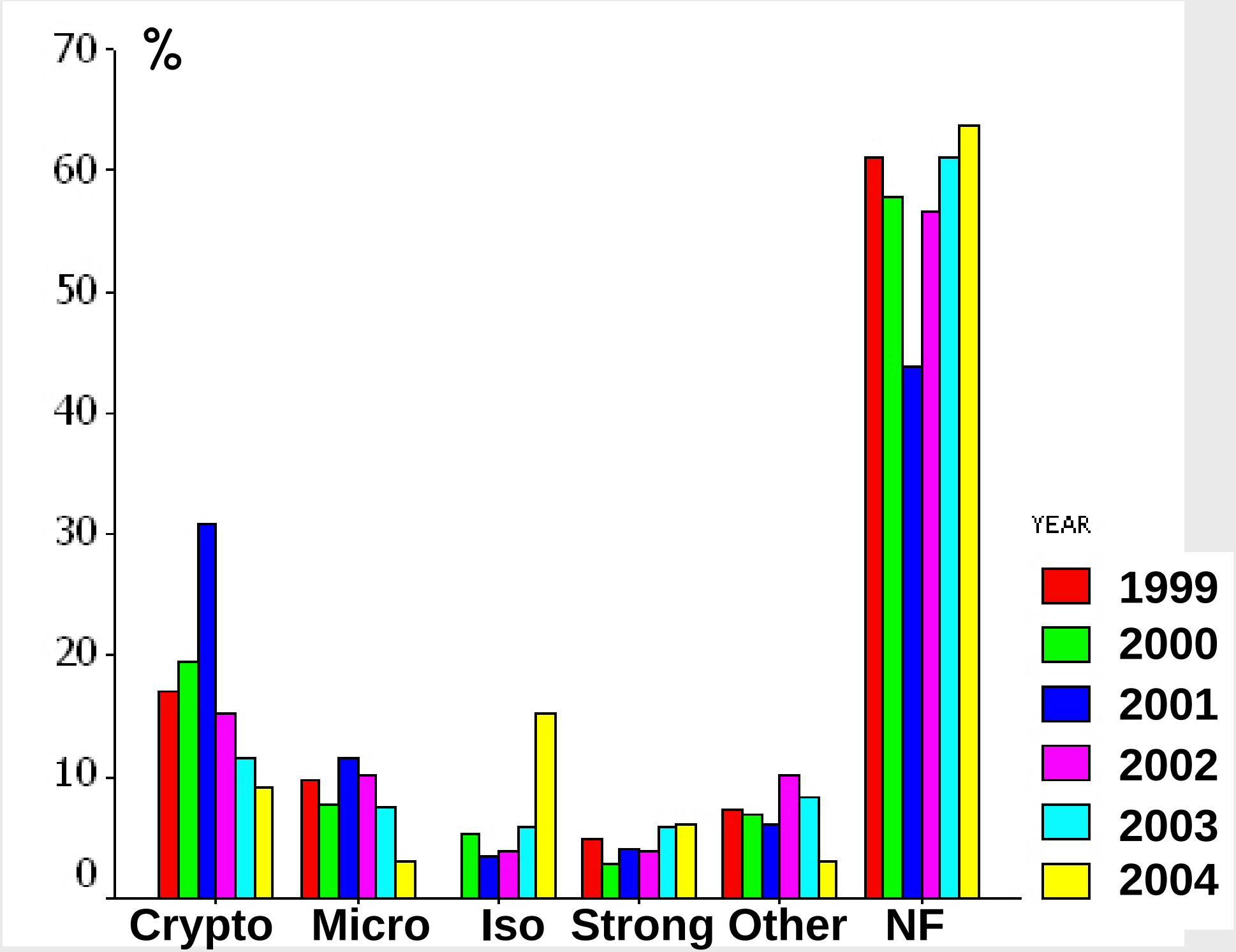
- Male : female = 2:1
- Median age was 34 (range 15 to 67) years.
- CD4 was measured in 334 patients
  - Median CD_4 count was 25 cell/cu.mm.
    (range 1 - 714)
  - 65.2% of them had CD4 count < 50 cell/cu.mm.
- Overall protozoan or helminthic infection were found in 432 (47.5%) patients.
- Dual pathogens were found in 64 (7%)patients

Protozoan pathogens identified in the study group (909 patients)

• *Cryptosporidial* oocyst	193 (21.5%)
• *Microsporidial* spore	101 (11.1%)
• *Isospora* oocyst	49 (5.4%)
• *Giardia lamblia*	30 (3.3%)
• *Entamoeba histolytica*	4 (0.4%)
• *Cyclospora*	5 (0.5%)
• *Strongyloides stercoralis*	40 (4.4%)

Bacterial Pathogens identified in a subgroup of 288 patients

Clostridium difficile	16 (15.6%)
Mycobacteria	18 (12.9%)
Salmonella spp.	11 (4.4%)
Campylobacter spp.	18 (7.1%)
Shigella spp.	1 (0.4%)
Total	64 (22.2%)


%
70
60
50
40
30
20
10
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Crypto
Micro
Iso
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Other
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YEAR
1999
2000
2001
2002
2003
2004

RESULTS

	Cryptosporidium-infected group	Microsporidium-infected group	Isospora-infected group	Controls	P-value
Total number	160	76	41	478	
Male,n (%)	85 (53.1)	46 (60.5)	28 (68.3)	314 (765.7)	0.03
Median(range) age, yrs	34 (21- 58)	32 (20-48)	36 (25-55)	34 (15-67	0.18
Median (range)CD4, cell/ml	11 (1-554)	20 (4-707)	46 (6-601	44 (1-714)	<0.001
- CD4<50cell/ml, n/total (%)	73/83 (88)	26/40 (65)	8/13 (61.5)	78/154 (50.6)	<0.001
Diarrhea, n/total (%)					
- No	6/136 (4.4)	4/68 (5.9)	0/36	40/371 (10.8)	<0.001
- less than 7 days	30/136 (22.1)	11/68 (16.2)	10/36 (27.8)	135/371 (36.4)	
- between 7-30 days	24/136 (17.6)	7/68 (10.3)	3/36 (8.3)	54/371 (14.6)	
- more than 30 days	76/136 (55.9)	46/68 (67.6)	23/36 (63.9)	142/371 (38.3)	
Weight lost, n/total (%)	107/124 (86.3)	47/61 (77)	27/31 (87.1)	258/352 (73.3)	0.01
Outcome					
- Dead, n/total (%)	68/160 (42.5)	9/76 (11.8)	0/41	17/478 (3.6)	<0.001

Genotyping : 34 samples

- ***C. hominis* (*C. parvum* human genotype, 50%)**
- ***C. meleagridis* (20%)**
- ***C. parvum* (15%)**
- ***C. felis* (4%)**
- ***C. canis* (4%)**

(using RFLP and sequencing of 18sRNA gene)

Gatei W, et al. Ann Trop Med Parasitol 2002

Human Cryptosporidiosis

Location	Type of patients	Total no. of patients	No. of patients infected with:				
			C. hominis	*C. parvum*	*C. meleagridis*	*C. felis*	*C. canis*
Portugal	HIV+	29	7	16	3	3	0
Switzerland	HIV+	13	2	7	1	3	0
France	HIV+	46	14	22	3	6	0
Thailand	HIV+	29	24	0	3	1	0
Thailand	HIV+	34	17	5	7	3	2
Atlanta	HIV+	10	5	1	0	3	1
New Orleans	HIV+	29	18	8	0	3	0
Peru	HIV+	118	76	20	10	4	9
Peru	Children	83	65	8	7	1	2
Kenya	All	33	23	8	1	0	0
Japan	All	22	16	3	3	0	0
United Kingdom	All	1,680–2,057	815	1,247	19	4	1

[a] Only data from studies using PCR that amplifies all five *Cryptosporidium* spp. are quoted.

Xiao L et al. Clin Microb Rev 2004; 17: 72- 97

A Randomized Placebo – Controlled Trial of Nitazoxanide for the Treatment of Cryptosporidiosis: Nov 1999- June 2004

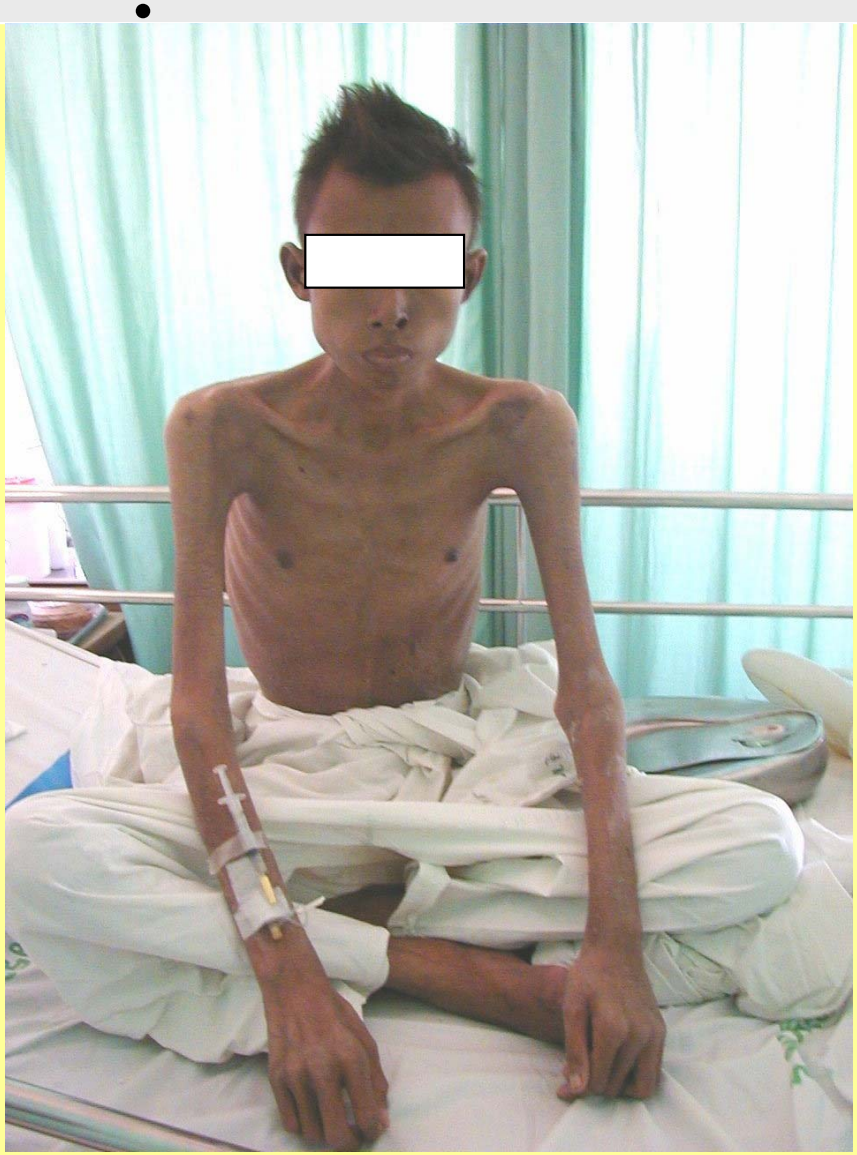
RESULTS

- 50 patients were studied
  - 37 patients were enrolled by Jan 2002
  - 11/37 patients died from advance AIDS.
- Overall 19 (32%) patients died,
- 6 (12%) patients lost to follow up.

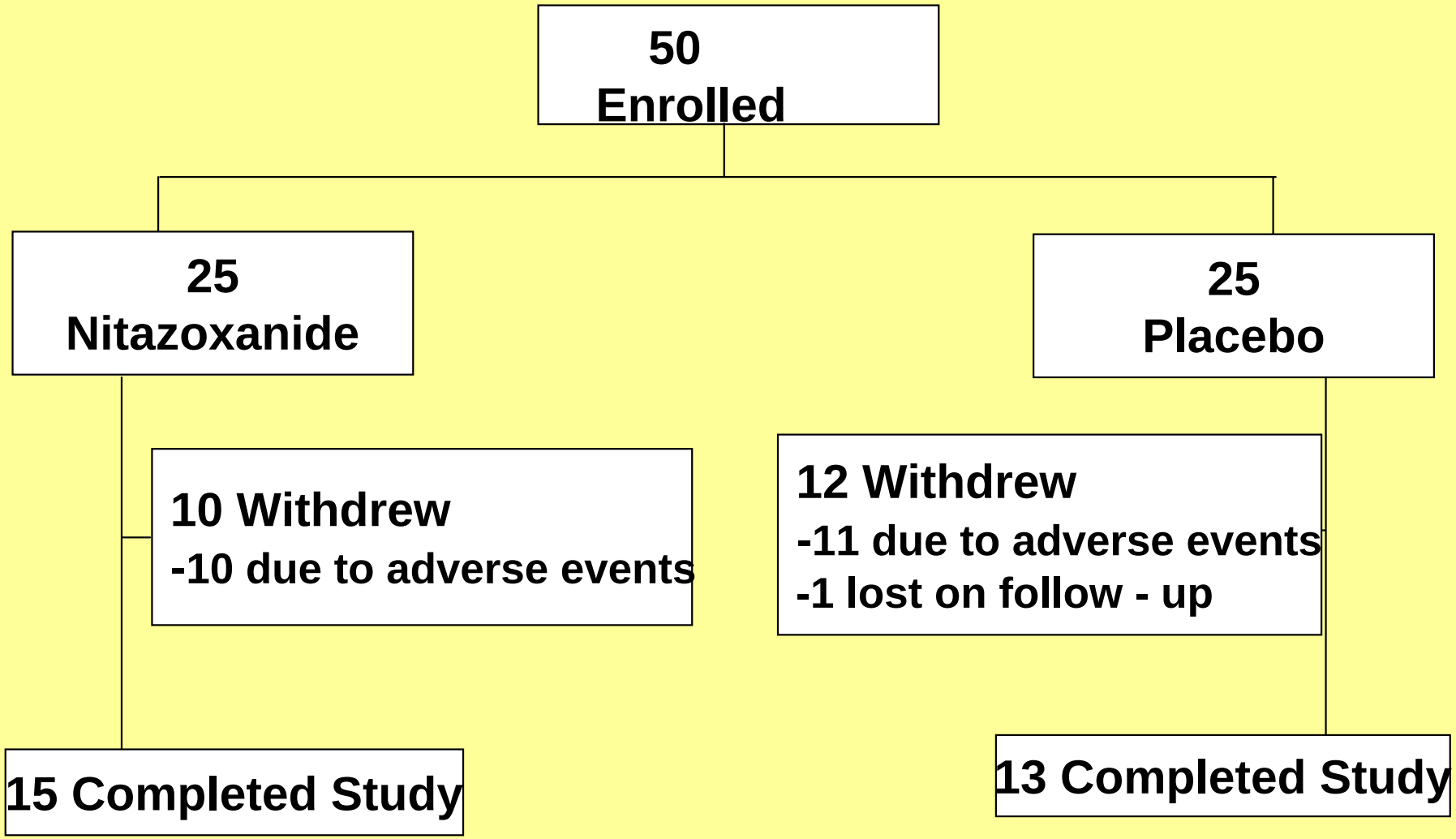
Outcome : Up to Jan 2002

- **Dead**
  - **Leukemia, lymphoma** **2**
  - **severe wasting** **7**
  - **MAC septicemia** **1**
  - **Cryptococcal septicemia** **1**
- **On ARV (DDI+ D4Tor D4T+3TC+ nevirapine)**
  - **improved** **8**
  - **failure** **2**
  - **initial therapy** **2**

Cryptosporidiosis



Patient Disposition Flow Chart



Demographic and Disease–Related Characteristics

	Total	Nitazoxanide	Placebo
Male: Female	20:21	9:13	11:8
Age, yr Mean (SD) Range	 34.3 (7) 21-62	 33.8 (6) 23-46	 34.4 (9) 21-62
CD4 count, cu.mm. Mean (SD) Range	 9.4 (6.8) 2-31	 10.9 (7) 3-31	 7.7 (6) 2-24
Duration of Diarrhea, d Median (range)	 90 (21-720)	 90 (21-720)	 90 (21-450)
Oocyst count, n - Many - Moderate - Few - Rare	 27 6 3 5	 13 4 2 3	 14 2 1 2

Results

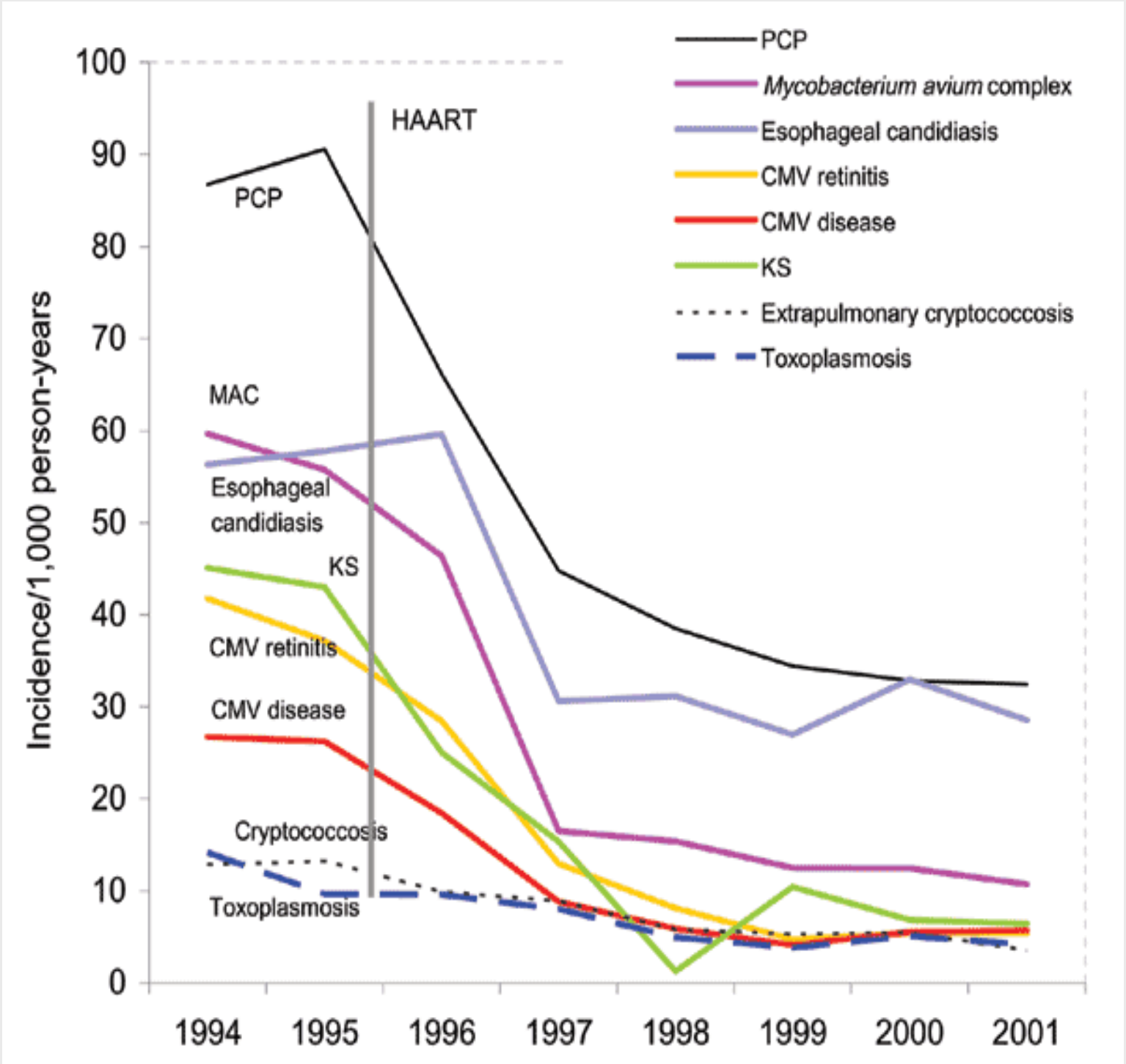
	Nitazoxanide n (%)	Placebo n(%)	P-value
•Clinical response			0.049
– Well	7 (32)	1 (5)	
– Continuing illness	15 (68)	18 (95)	
• Parasitological response			0.49
– Eradication	2 (9)	0 (0)	
– Persistence	20 (91)	19 (100)	
• Therapeutic response			0.49
– Cure	2 (9)	0 (0)	
– Failure	20 (91)	19 (100)	

Adverse Events (AE)

- **48/50 patients reported AE**
  - **86 AE in nitaxozanide group and 78 AR in placebo group**
  - **Most AEs were not related to the drug except mild or transient yellowish sclera in 6/25 nitaxozanide group**
- **6 deaths in nitazoxanide and 5 deaths in placebo group**

Open Label Study

- **14 patients were entered the study**
  - **10/14 showed “well” clinical responses**
  - **1/14 absent of cryptosporidium oocyst but relapse at the end of 8 week treatment**



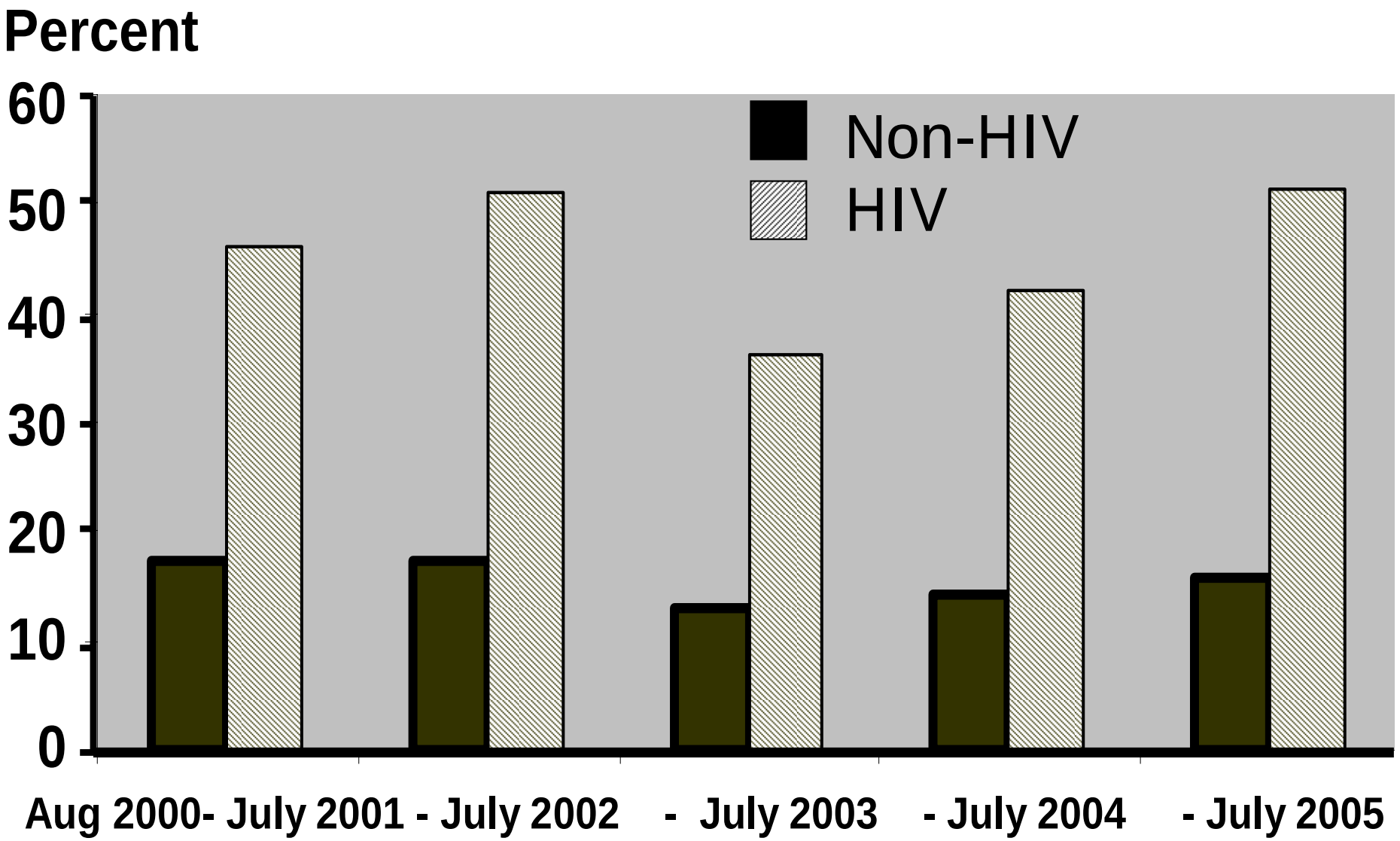
Morris et al.

EID 2004

Opportunistic protozoa in stool samples from HIV-infected patients

- 22 HIV-infected patients with chronic diarrhea (>3 weeks)
  - Microsporidia 6 (27.3%)
  - Cryptosporidia 2 (9%)
  - Isospora belli 1 (4.5%)
  - Giardia intestinalis 2 (9.1%)
  - Candida spp. 7 (31.8%)
  - Strongyloides stercolaris 3 (13.6%)
  - Opisthorchis viverrini ova 1 (4.6%)

Punpoowong et al. 1998



%Intestinal Parasite in Non-HIV and HIV Infected Patients

Intestinal Parasitic Infection: Siriraj Hospital (1999-2005)

- **The overall prevalence was 18.9%**
- **Infection rate was 44.9% in HIV &15.6% in non-HIV patient**
- **Helminthic infection was found in 8.9% of both groups.**
- **The Prevalence of protozoan infection was 29.6% in HIV group versus 3.9% in non-HIV group**
- **The prevalence of *Cryptosporidium spp*. was 20.7%, and**
- **The prevalence of microsporidial infection was 15.5% in HIV patients**

Prevalence of *Cryptosporidium* in Thailand : 2007

- 46 HIV patients from Prabat Numpu Temple, Lopburi, Thailand
- The prevalence was 28.3% (13/46)
- 5/13 (15.1%) of patients with diarrhea and 8/33 (24.2%) of patients without diarrhea
- Four isolates were confirmed to be *C. parvum* by genotyping

Nuchjangreed C. et al Parasitol Res.2008

Conclusion

- ***Cryptosporidium spp*. remains a significant intestinal protozoan in HIV –infected patients in Thailand.**
- **Immune reconstitution is the key to eradication and prevention of cryptosporidiosis among these HIV-infected patients.**

Conclusion

- **Nitazoxanide is effective against cryptosporidiosis, but only in patients with CD4 >50**
- **The efficacy of nitazoxanide was not significant different from placebo in this small study of patients with CD4 <50.**
- **High dose, and 8 week duration of treatment was well tolerated.**

Conclusion

- **In patients unable to take ARV, cryptosporidium diarrhea remains a challenging disease.**
- **More studies such as *Cryptosporidium* genome will assist to the discovering of new gene, biochemical pathways and protective antigens that can be targeted to develop novel therapies for cryptosporidiosis.**

Acknowledgement

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