Prevalence of enteric viruses among hospital patients with AIDS in Kinshasa, Zaire

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Abstract
Diarrhoea is the most common manifestation of acquired immunodeficiency syndrome (AIDS) in Africa. Numerous parasitic or bacterial agents have been implicated, but a pathogen-specific aetiology has not been found. Enteric viruses (i.e., rotavirus, small round structured viruses, coronavirus, and adenovirus) were detected by enzyme-linked immunosorbent assay or electron microscopy in faecal specimens of 17% of 198 consecutive adult admissions to a general medical ward of an urban hospital in Kinshasa, Zaire. Overall, 57% of patients were seropositive for infection with human immunodeficiency virus (HIV) 1; of these, 50% were classified as World Health Organization AIDS stage IV. The prevalence of enteric viruses in stool specimens did not differ significantly between patients with and without HIV infection, and was not associated with acute or chronic diarrhoea, or constitutional symptoms. However, a trend ($P=0.14$) towards greater frequency of virus in stools from patients in the lower 3 quintiles of the CD4/CD8 T cell ratio was seen. This trend approached statistical significance ($P=0.07$) with stratification by HIV infection. Although we found no evidence in this population to support a major pathogenic role for these viruses alone in the enteropathy of AIDS, increased viral shedding was weakly associated with immunodeficiency.

Introduction

Chronic diarrhoea is a common manifestation of acquired immunodeficiency syndrome (AIDS) in African (COLEBUNDERS et al., 1987a) and western countries (MODIGLIANI et al., 1985). As many as 40% of AIDS patients in Africa develop a syndrome of chronic diarrhoea and wasting which, in most cases, rapidly leads to death (COLEBUNDERS et al., 1988). The cause of prolonged diarrhoea in these patients is largely unknown since an infectious aetiology has been found in only a small proportion of patients who have been systematically studied for bacteria and parasites. *Cryptosporidium parvum* and *Isospora belli* are the most common infectious agents found (MODIGLIANI et al., 1985) but account for less than half of all cases (COLEBUNDERS et al., 1988; SEWANKAMBO et al., 1987).

The role of pathogenic enteric viruses in AIDS-associated diarrhoea remains unclear. Recently, studies in Australia and the United States have identified several groups of enteric viruses present in the stool of homosexual men infected with human immunodeficiency virus (HIV-1) with and without AIDS (CUNNINGHAM et al., 1988; KALJOT et al., 1989). Although these agents were implicated as possible causes of acute or relapsing episodes of diarrhoea, they were not associated with the presence of chronic diarrhoea. Specimens from symptomatic HIV-infected African patients, to our knowledge, have not previously been examined for the presence of enteric viruses. We report here the results of a pilot cross-sectional survey of 198 unselected consecutive admissions to a large hospital in Kinshasa, Zaire, to determine the prevalence of enteric viruses and their relation to diarrhoea, wasting and immunosuppression among HIV-infected and uninfected patients in an area where diarrhoeal disease is common and heterosexual HIV transmission predominates.

Subjects and Methods

Patients

Mama Yemo Hospital is a 2000-bed general hospital located in central Kinshasa, Zaire, that provides medical, surgical, paediatric, gynaecological, and obstetric care to a large urban and predominantly indigent population. From July to October, 1989, 234 consecutive admissions to one male and one female adult general medical pavil-

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lion were systematically evaluated by a study physician after informed consent was obtained. Of these, 36 patients were excluded from the analysis because they appeared moribund, died within 24 h of admission, or refused to co-operate. A comprehensive medical history and physical examination were performed on each patient. HIV serological testing and a confirmatory Western blot were performed on sera from all patients. A diagnosis of AIDS was based on HIV-1 seropositivity and World Health Organization (WHO) clinical criteria for AIDS in Africa (WHO, 1990; COLEBUNDERS et al., 1987b). A single stool specimen was obtained within 48 h of admission to hospital from the 198 assessable patients and blood was taken for T cell subset typing. Patients were monitored throughout their hospital stay, and the final diagnosis and disposition were recorded. Acute diarrhoea was defined as the presence of at least 3 stools that were looser than usual during a 24 h period and chronic diarrhoea was defined as the presence of loose stool for more than 2 out of 4 weeks.

Serology

Serum was tested for antibody to HIV by an enzyme linked-immunosorbent assay (ELISA) (Organon Teknika BV, Boxtel, The Netherlands). A confirmatory Western blot assay (DuPont de Nemours, Wilmington, Delaware, USA) was performed on all serum samples positive by ELISA and was considered positive when antibody to protein bands p24 and gp120/160 was detected.

T lymphocyte phenotyping

Freshly drawn whole blood samples were stained with fluorescein-labelled monoclonal antibodies (Leu-2 and Leu-3, Becton Dickinson, Antwerp, Belgium) directed against CD4+ and CD8+ lymphocyte antigens and counted by a fluorescence-activated cell analyser (Hewlett-Packard).

Stool examination

Stool samples were examined for the presence of the coccidial pathogens, *I. belli* and *C. parvum*, by fluorescence microscopy using a rhodamine-auramine stain and confirmed with a modified Kinyoun's acid-fast stain. Unconcentrated fresh stool specimens were stained with rhodamine-auramine and examined for coccidial parasites for a minimum of 5 min each.

Enteric virus identification

Stool samples were stored in sealed containers under

vegetable oil at 4°C until transported to the Centers for Disease Control (CDC) for examination. Direct electron microscopy (EM) was performed using a concentration technique described previously (CUNNINGHAM et al., 1988). In brief, refrigerated specimens (1 g) were prepared as a 10% suspension in phosphate-buffered saline (PBS). This was extracted with an equal volume of Genetron® (1,1,2,trichlorotrifluoroethane), and the supernatant was centrifuged (100 000 g for 1 h). The pellet was resuspended in 0·5 ml PBS and 20 µl were floated on a 400-mesh Formvar® carbon-coated grid for 1 min. The grid was stained with phosphotungstic acid (pH 7·9) for 45 s and examined in a Phillips 201 electron microscope.

Aliquots of the 10% suspension were tested by ELISA for rotavirus of groups A and B and for adenovirus hexon using methods previously developed at CDC and described elsewhere (FANG et al., 1989; LEW et al., 1991). Specimens positive for adenovirus hexon were serotyped by using a commercial monoclonal antibody assay to identify enteric serotypes 40 and 41 (Cambridge Bio-Science).

Statistical analysis

Dichotomous variables were compared by χ^2 analysis. CD4/CD8 ratio quintiles were evaluated by 2×5 contingency tables. Expected frequency remained less than 50% in all cells. Two-tailed P values were used throughout and the accepted level of significance was $\leq0·05$.

Results

Of the 198 patients evaluated in this study, 57% were HIV-positive and 50% of these had advanced stage IV disease. Overall, 34 (17%) of the 198 specimens had one or more of the 4 types of enteric viruses (Table 1) of

Table 1. Presence of acute or chronic diarrhoea in patients shedding enteric virus

	Diarrhoea			
	Acute	Chronic	No diarrhoea	Total
No. examined	30	37	131	
Enteric virus				
Rotavirus	2 (6·6%)	4 (10·8%)	14 (10·7%)	20
SRSV[a]	2 (6·6%)	2 (5·4%)	6 (4·6%)	10
Coronavirus	1 (3·3%)	0	3 (2·3%)	4
Adenovirus	0	1 (3·3%)	1 (0·8%)	2
Total	5 (16·0%)	7 (18·9%)	24 (18·3%)	36[b]

[a]Small round structured viruses.
[b]Two double infections with rotavirus and coronavirus.

which rotavirus (group A) was the most frequent. All rotaviruses and adenoviruses (serotypes 40 and 41) detected by ELISA were confirmed by direct EM. Viral shedding did not differ significantly between males (16% of 96 males) and females (19% of 102 females) or among age quartiles. Double infection with rotavirus and coronavirus was found in 2 HIV seronegative women with no history of diarrhoea.

The distribution of enteric viruses among the patients according to HIV serostatus and clinical stage of HIV disease are shown in Table 2. Nearly all (93%) of the seropositive patients were in the advanced stages III or IV of their HIV disease and 49% died during their stay in hospital. The HIV negative patients were also extremely ill with an in-hospital death rate of 22%. The prevalence of viral shedding was 17% overall, with an equal distribution between HIV-positive (17%) and HIV-negative (18%) patients ($P=0·82$). Immunocompromise, indicated by advanced clinical HIV stage, was not associated with increased viral shedding. Although there was no significant difference in viral shedding among the 4 HIV stages, there were relatively few patients in the less immunocompromised stages I and II (Table 3). Viral shedding was weakly associated with more advanced immunoincompetence, as measured by the ratio of circulating CD4 and CD8 T cells (Table 4). When only HIV infected patients were considered the trend was stronger ($P=0·07$). Viral excretion in the stool was not associated with a history of recent, or a report of present, acute or chronic diarrhoea (Table 1), fever, vomiting, or significant ($>10\%$) weight loss at the time of the study (data not shown). Diarrhoeal stool which contained virus was no more likely to contain blood, pus, or mucous than if viral shedding was absent (data not shown). Nonetheless, chronic diarrhoea was highly correlated with HIV seropositivity ($P<0·01$), HIV stage (Mann-Whitney $P<0·001$) or CD4/CD8 T-cell ratio (Mann-Whitney $P<0·01$), although acute diarrhoea was not.

I. belli or _C. parvum_ were detected in the stool of 35 patients and the presence of one or other was highly correlated with HIV serostatus (80% vs. 20%, $P=0·005$). In seropositive patients the presence of coccidia was weakly associated with acute diarrhoea (29% vs. 14%, $P=0·08$) but not with chronic diarrhoea (27% vs. 24%, $P=0·77$). Because these organisms may produce diarrhoea they could potentially confound any association between the presence of enteric virus and acute or chronic diarrhoea. However, analysis after removal of patients with faecal coccidia did not reveal any significant association between enteric virus and diarrhoeal symptoms, HIV serostatus, HIV stage or CD4/CD8 ratio.

Discussion

In this study, we found antigenic and/or EM evidence of enteric viruses in the stool of 17% of adult patients who were recently admitted to the general medical wards of Mama Yemo Hospital in Kinshasa, Zaire. Equal detection rates were seen among patients with and without acute or chronic diarrhoea. While 17% appears to be a relatively high prevalence rate of viral carriage for adults, it is notable that CUNNINGHAM et al. (1988) found a similar rate in their group of HIV-negative and healthy HIV-positive homosexual adult men attending a sexually transmitted disease (STD) clinic in Australia. The patients reported by CUNNINGHAM et al. (1988) and those

Table 2. Distribution of enteric viruses among patients grouped by HIV serostatus and WHO clinical HIV stage

HIV serostatus and stage[a]	No.	Rotavirus	SRSV[b]	Coronavirus	Adenovirus	Any virus
Positive						
I	5	2	–	–	–	2 (40%)
II	3	1	–	–	–	1 (33%)
III	56	3	1	2	–	6 (11%)
IV	50	5	4	–	1	10 (20%)
Total	114	11	5	2	1	19 (17%)
Negative	84	9[c]	5	2[c]	1	15 (18%)

[a]See WHO (1990) for specific criteria. Stage I, asymptomatic/PGL; stage II, mild disease; stage III, moderate disease; stage IV, AIDS.
[b]Small round structured viruses.
[c]Two double infections with rotavirus and coronavirus.

Table 3. Mean CD4+/CD8 cell ratio in selected patient groups with and without enteric virus

Group	No.	Enteric viruses	Cell ratio[a] No.	No enteric viruses	P
HIV negative	11	1·64±1·03	55	1·56±0·69	0·76
HIV positive	15	0·34±0·32	75	0·37±0·50	0·82
Stage I[b]	1	0·44	1	0·40	–
Stage II[b]	1	0·88	2	0·42±0·22	–
Stage III[b]	5	0·37±0·36	39	0·43±0·56	0·80
Stage IV[b]	8	0·25±0·27	33	0·29±0·43	0·79

[a]Mean CD4+/CD8 cell ratio±standard deviation.
[b]See WHO (1990) and footnote to Table 2.

Table 4. Numbers of patients from whom enteric virus was isolated according to CD4/CD8 quintile, stratified by HIV infection

Quintile	CD4/CD8 ratio	No. of patients from whom enteric virus was isolated Overall ($n=156$)	HIV positive ($n=90$)	HIV negative ($n=66$)
1	<0·08	5/31	5/31	0/0
2	0·08–0·42	4/31	3/29	1/2
3	0·42–0·95	10/33	7/19	3/14
4	0·95–1·60	2/30	0/9	2/21
5	>1·60	5/31	0/2	5/29
		$P=0·14$[a]	$P=0·07$[a]	$P=0·45$[a]

[a]Calculated by χ^2.

included in this study were both at increased risk of exposure to enteric viruses; homosexual STD clinic attenders because of oral-anal sexual practices and the patients reported here because of crowded living conditions and close proximity to young children who could serve as viral reservoirs. The fact that patients admitted to Mama Yemo Hospital tend to be very sick (overall in-hospital mortality rate=41%) and frequently have advanced malnutrition impairing their resistance to infection may also account for the high rate of viral shedding reported here. Although the possibility exists that this high prevalence rate represents some nosocomial transmission, this is unlikely because most stools were obtained on the day of admission and all were obtained within 48 h of admission. Seroepidemiological studies in non-industrialized countries indicate that exposure to rotavirus and the Norwalk family of viruses occurs during early childhood, and that by adulthood over 70% of the population have been exposed (GREENBERG et al., 1979). Additionally, prospective studies have shown that serological immunity in adults is not universally protective and that adults may become reinfected, albeit with mild or no symptoms (WENMAN et al., 1979). Indeed, in a non-prospective study of rotaviral infection in adult contacts of paediatric patients, 88% were asymptomatic (KIM et al., 1977). Thus, the high detection rates of enteric viruses in patients without diarrhoea reported here may indicate that hyperendemic enteric viral infection existed in this population.

The role of enteric viruses in the pathogenesis of chronic diarrhoea associated with AIDS is unknown. Severe or chronic enteritis due to rotavirus, adenovirus, or coxsackievirus has been reported in bone marrow transplant recipients (SAULSBURY et al., 1980; YOLKEN et al., 1982), suggesting that T cell competence may be important in the host's defence against these agents or in the termination of the diarrhoeal event following infection. Alternatively, the local mucosal effects of intensive chemotherapy, large doses of radiation used in bone marrow transplantation, or graft-versus-host disease may play a more significant role in disrupting host defence against enteric viruses than alterations in cellular immunity. In this regard, it is notable that, despite finding increased carriage of enteric viruses among homosexual patients with more advanced HIV disease, CUNNINGHAM et al. (1988) could not demonstrate an association with

acute or chronic diarrhoea. Although they reported that the rate of viral shedding was increased to 50% in patients with symptomatic HIV disease compared with a background of 18% in asymptomatic HIV infected patients, they could not show a difference in the frequency of either acute or chronic diarrhoea. KALJOT et al. (1989) reported a lower prevalence (9%) of enteric viruses in homosexual patients in the United States but also found viral shedding more frequently among homosexual patients with AIDS than among homosexual patients with AIDS-related complex. Like CUNNINGHAM et al. (1988), KALJOT et al. (1989) found no correlation between viral shedding and the presence of diarrhoea in any group. In contrast, SMITH et al. (1988) reported no evidence of rotavirus infection in 32 homosexual AIDS patients in the United States with or without diarrhoea, but did not test for other enteric viruses. Regardless of HIV serostatus or HIV-associated symptoms, we did not find an association between gastrointestinal virus in the stool and the presence of acute or chronic diarrhoea or constitutional symptoms. This could be due to the lack of a healthier non-diarrhoeal control group, the high asymptomatic carriage rate in this population, or the cross-sectional nature of the study. Our results suggest that enteric viruses do not play a pathogenic role in diarrhoea in the populations reported here, although the sample size of our study may have been too small to show an association at the relatively low detection rates found for the individual viral agents. Given the high virus detection rates in non-diarrhoeic patients, further studies to assess a causal association for any of these enteric viruses would require prospective study groups of more than 150 cases and an equal number of controls to detect a 40% difference in rates of infection between groups with and without diarrhoea.

Because coccidial enteric infection is implicated in the largest proportion of diarrhoeal cases in AIDS patients from non-industrialized areas (COLEBUNDERS et al., 1988; DEHOVITZ et al., 1986), we sought to identify and exclude patients who may have been symptomatic due to these infections. Thus, hidden associations between enteric viruses and diarrhoeal symptoms are less likely to be obscured by excluding cases of potential coccidial diarrhoea. For completeness, data presented in the Tables includes the 35 cases with coccidial infection but analysis after the removal of these cases did not reveal any hidden association. Because we did not perform routine bacterial stool cultures we cannot exclude any cases of bacterial diarrhoea that may have occurred and, therefore, possibly obscured an association.

Although we could not confirm an increased rate of viral shedding associated with T cell immunosuppression, there was a weak trend towards identification of enteric virus among patients with CD4/CD8 T cell ratio in the lower quintiles ($P=0·14$). This did not achieve statistical significance but the trend became stronger when stratified by HIV infection status (Table 4). In spite of meaningful comparisons between early and advanced HIV disease being limited because of the few patients with mild HIV disease in this study, we believe that comparisons of immunocompetence based on

CD4/CD8 ratios allowed us to use HIV-uninfected patients as adequate controls. Despite the high mortality (22%) and debilitated state of these HIV-negative patients, their mean CD4/CD8 ratio was normal (Table 3). Given this, we were able to demonstrate that a low CD4/CD8 ratio was weakly associated with a greater probability of passing enteric virus, regardless of whether diarrhoeal disease was present or not. This was similar to the results of CUNNINGHAM et al. (1988) and KALJOT et al. (1989), who reported that the presence of enteric virus in the stool of HIV-infected homosexual patients was related to the degree of immunosuppression, determined by advancing clinical HIV stage. Neither symptoms or infection correlated very well with the presence of serum antibodies to enteric viral infections (GREENBERG et al., 1979; KAPIKIAN et al., 1983) and the data presented here suggest that cellular immunity may play a role in this interaction. Further studies that address the role of secretory immunity as well as local and systemic cellular immunity are needed.

In conclusion, these cross-sectional data indicate that enteric viruses are present in a substantial proportion of hospital patients in Kinshasa, Zaire, and that they are not a significant cause of diarrhoea in African AIDS patients. Other aetiologies of chronic diarrhoea and wasting, such as small bowel overgrowth and tropical sprue, need to be examined as potential causes of chronic diarrhoea in these patients. A trend towards greater viral shedding with advanced immunosuppression was detected.

References
Colebunders, R., Mann, J. M., Francis, H., Bila, K., Izaley, L., Kakonde, N., Kabasde, K., Ifoto, L., Nziambi, N. & Quinn, T. C. (1987a). Evaluation of a clinical case-definition of acquired immunodeficiency syndrome in Africa. Lancet, i, 492–494.

Colebunders, R., Francis, H., Mann, J. M., Bila, K., Izaley, L., Kimputu, L., Behets, F., van der Groen, G., Quinn, T. C. & Curran, J. W. (1987b). Persistent diarrhea strongly associated with HIV infection in Kinshasa, Zaire. American Journal of Gastroenterology, 82, 859–864.

Colebunders, R., Lusakamunu, K., Nelson, A. M., Gigase, P., Lebughe, I., van Marck, E., Kapita, B., Francis, H., Salaun, J. J. & Quin, T. C. (1988). Persistent diarrhea in Zairian AIDS patients: an endoscopic and histological study. Gut, 29, 1687–1691.

Cunningham, A. L., Grohmann, G. S., Harkness, J., Law, C., Marriott, D., Tindall, B. & Cooper, D. A. (1988). Gastrointestinal viral infections in homosexual men who were symptomatic and seropositive for human immunodeficiency virus. Journal of Infectious Diseases, 158, 386–391.

Dehovitz, J. A., Pape, J. W., Boncy, M. & Johnson, W. D. (1986). Clinical manifestations and therapy of Isospora belli infection in patients with acquired immunodeficiency syndrome. New England Journal of Medicine, 315, 87.

Fang, Z. Y., Ye, Q., Ho, M. S., Dong, H., Qing, S., Penaranda, M. E., Hung, T., Wen, L. & Glass, R. I. (1989). Investigation of an outbreak of adult diarrhea rotavirus in China. Journal of Infectious Diseases, 160, 948–953.

Greenberg, H. B., Valdesuso, J., Kapikian, A. Z., Chanock, R. M., Wyatt, R. G., Szmuness, W., Larrick, J., Kaplan, J., Gilman, H. & Sack, D. A. (1979). Prevalence of antibody to the Norwalk virus in various countries. Infection and Immunity, 26, 270–273.

Kaljot, K. T., Ling, J. P., Gold, J. W. M., Laughon, B. E., Bartlett, J. G., Kotler, D. P., Oshiro, L. S. & Greenberg, H. B. (1989). Prevalence of acute enteric viral pathogens in acquired immunodeficiency syndrome patients with diarrhea. Gastroenterology, 97, 1031–1032.

Kapikian, A. Z., Wyatt, R. G., Levine, M. M., Yolken, R. H., Van Kirk, D. H., Dolin, R., Greenberg, H. B. & Chanock, R. M. (1983). Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. Journal of Infectious Diseases, 147, 95–106.

Kim, H. W., Brandt, C D., Kapikian, A. Z., Wyatt, R. G., Arrobio, J. O., Rodriguez, W. J., Chanock, R. M. & Parrot, R. H. (1977). Human reo-virus like agent infection: occurrence in adult contacts of pediatric patients with gastroenteritis. Journal of the American Medical Association, 238, 404–407.

Lew, J. F., Moe, C. L., Monroe, S. S., Allen, J. R., Harrison, B. M., Forrester, B. D., Stine, S. E., Woods, P. A., Hierholzer, J. C., Herrmann, J. E., Blacklow, N. R., Bartlett, A. V. & Glass, R. I. (1991). Astrovirus and adenovirus associated with diarrhea in children in day care centers. Journal of Infectious Diseases, 164, 673–678.

Modigliani, R., Bories, C., Le Charpentier, Y., Salmeron, M., Messing, B., Galian, A., Rambaud, J. C., Leverque, A., Cochand-Priollet, B. & Desportes, I. (1985). Diarrhea and malabsorption in acquired immune deficiency syndrome: a study of four cases with special emphasis on opportunistic protozoan infestations. Gut, 26, 179–187.

Saulsbury, F. T., Winkelstein, J. A. & Yolken, R. H. (1980). Chronic rotavirus infection in immunodeficiency. Journal of Pediatrics, 97, 61–65.

Sewankambo, N., Mugerwa, R. D., Goodgame, R., Carswell, J. W., Moody, A., Lloyd, G. & Lucas, S. B. (1987). Enteropathic AIDS in Uganda. An endoscopic, histological and microbiological study. AIDS, 1, 9–13.

Smith, P. D., Lane, H. C., Gill, V. J., Manischewitz, J. F., Quinnan, G. V., Fauci, A. S. & Masur, H. (1988). Intestinal infections in patients with the acquired immunodeficiency syndrome (AIDS). Annals of Internal Medicine, 108, 328–333.

Wenman, W. M., Hinde, H., Feltham, B. & Gurwith, M. (1979). Rotavirus infection in adults: results of a prospective family study. New England Journal of Medicine, 301, 303–306.

WHO (1990). Acquired immunodeficiency syndrome (AIDS): interim proposal for a WHO staging system for HIV infection and disease. Weekly Epidemiological Record, 65, 221–224.

Yolken, R. H., Bishop, C. A., Townsend, T. R., Bolyard, E. A., Bartlett, J., Santos, G. W. & Saral, R. (1982). Infectious gastroenteritis in bone-marrow-transplant recipients. New England Journal of Medicine, 306, 1009–1012.