

## ETIOLOGICAL AGENTS OF DIARRHEA IN PATIENTS INFECTED BY THE HUMAN IMMUNODEFICIENCY VIRUS-1: A REVIEW

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### SUMMARY

Despite the importance of understanding the epidemiology of agents responsible for infectious diarrhea in human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) population, the number of articles about this subject is relatively few. The current article summarizes published data on bacterial, fungal, viral and parasitic enteropathogens in the HIV/AIDS seropositive subjects in different countries, regions and localities. In general, there is a great difference in the frequencies of etiological agents due to factors which include immune status, geographical location, climate and socioeconomic conditions. It is important to stress that a great prevalence of infection by emergent agents has been reported in the more advanced stages of AIDS. Therefore, to establish specific treatment depends directly on knowledge of these agents and risk factors associated to their distribution. Moreover, the colonization by potential pathogenic agents verified in these individuals is high thus implicating that they act as carriers. Finally, public health measures of control and prevention must take into consideration the regional previously identified enteropathogens, especially in areas where HIV prevalence is high.

**KEYWORDS:** Enteropathogens; Prevalence; Diarrhea; HIV; AIDS.

In spite of worldwide efforts and the promising advances aimed at the control of acquired immunodeficiency syndrome (AIDS), whether for prevention or treatment, the number of individuals infected by the human immunodeficiency virus (HIV) is growing as is the number of deaths related to this disease. In fact in 2006, 4.3 million adults and children were identified as newly infected throughout the world<sup>90</sup>. In Latin America, one third of HIV seropositive individuals are concentrated in Brazil, a country that mirrors this worldwide increase<sup>10,91</sup>.

Superimposed infections due to defective immunity are a major health problem among HIV seropositive patients. Intestinal pathogen infection, which is also one of the basic health problems in tropical regions, is common in these patients<sup>55</sup>. Among the possible clinical manifestations of AIDS, diarrhea represents a great concern<sup>20</sup> as this group is susceptible to both classic pathogens and different opportunistic agents<sup>16-18,61,65</sup> malnutrition and, ultimately, death may be associated to these co-infections<sup>92</sup>. Furthermore, aggravating this condition, antibacterial chemoprophylaxis, commonly prescribed to this group of patients, causes severe damage to gastrointestinal flora<sup>72</sup>. Therefore, diarrhea is being considered a significant cause of morbidity and mortality among HIV/AIDS patients. Indeed, this is the second most common diagnosis and, in developing countries, affects approximately 90% of patients with AIDS<sup>4,26,55,69</sup>.

In addition, diarrhea in patients with HIV infection is a big challenge

because, apart from etiologies common to the general population, one must consider non-infectious clinical manifestations due to the well-known adverse reaction to antiretroviral drugs<sup>11,44,80,95</sup>. However, non-infectious diarrhea, for the majority of patients, is limited to a period of one to two months<sup>70</sup>. On the other hand, there are those who require greater medical attention since low TCD4<sup>+</sup> cell counts ( $\leq 200$  cells/mm<sup>3</sup>) cause greater susceptibility to the infectious form<sup>22,95</sup>.

Due to the importance of knowledge on microorganisms implicated in infectious diarrhea and on the individual and collective impact of infection, there is a need for more publications on this subject<sup>93</sup>. This fact becomes more evident when it comes to the HIV/AIDS group, in which the majority of publications are on the enteroparasites infections<sup>96</sup>. This article aims to summarize published data on bacterial, fungal, viral and parasitic enteropathogens in HIV/AIDS populations from different countries, regions and localities since the broad socioeconomic, climatic and geographic diversity is known to impact their significance<sup>18,78</sup>.

### Bacteria

Although the isolation of *Enterobacteriaceae* from the gastrointestinal tract of immunocompromised individuals has been interpreted as colonization due to the absence of clinical signs, characterizing an asymptomatic carrier, infectious diarrhea by this group of microorganisms does frequently occur. Bacteria usually isolated in the population are

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the same detected in the group of immunocompetent: *Escherichia coli*, *Salmonella* sp. and *Shigella* sp.<sup>71,95</sup>. Nevertheless, traditional diagnostic methods are in general limited on the isolation and identification of opportunistic or emerging bacteria<sup>93</sup>.

Several strains of the *E. coli* species described as important pathogens (*E. coli* enterotoxigenic-EPEC, *E. coli* enteroaggregative-EAEC, *E. coli* diffusely adherent-DAEC, *E. coli* enteroinvasive-EIEC, and *E. coli* Enterohemorrhagic-EHEC) are responsible for about 25% of acute diarrhea in immunocompetent individuals<sup>71</sup>. In HIV seropositive patients, this species has been strongly associated with persistent diarrhea with a great diversity of virulence factors and consequent effects. In Africa, GASSAMA-SOW *et al.*<sup>37</sup> found a positive correlation between chronic diarrhea and the isolation of *E. coli* from the intestinal mucosa of AIDS patients. However, in Brazil this association was not seen in a HIV-infected infant population in which similar frequencies of colonizing *E. coli* were found in diarrheic and non-diarrheic children<sup>78</sup>. Other authors associated EAEC with diarrhea severity (intestinal inflammation and blood in stools) and a higher isolation frequency (70%) among AIDS patients in comparison to the HIV seronegative population<sup>85,94</sup>.

There seems to be no differences related to the immune condition and the commonest isolated species of the *Shigella* genus<sup>6</sup>, although an approximately twenty-fold increase in risk to develop gastroenteritis has been described in AIDS patients carrying this bacterium compared to the general population<sup>35,36</sup>. Recently, *S. sonnei* was detected in 11% of diarrheic HIV-infected children from the Northwestern region of São Paulo State, Brazil<sup>78</sup>, but without a positive association with this condition. Approximately the same percentage of *Shigella* sp. was reported for adult HIV seropositive diarrheic individuals from Southern India<sup>54</sup>. *Salmonella* sp. can be considered another important pathogen for the HIV-infected group as these enterobacteria are 20 to 100 times more frequently isolated among HIV individuals compared to the immunocompetent population<sup>13,60</sup>. The frequencies of *Salmonella* isolation varies from 2.5% to 18% with the highest percentages in African children<sup>2,54</sup>. A special mention must be made to non-typhoid salmonella because of its severe, invasive and recurrent pattern in HIV-infected individuals<sup>2,19,52</sup>. Enteric salmonellosis was also more frequently seen in patients in the advanced stages of HIV infection and with impaired nutritional status<sup>86</sup>.

Among enteric bacterial pathogens, *Campylobacter jejuni* has been detected in significantly higher frequencies among HIV positive populations<sup>54</sup>. In countries of South Asia and on the African continent, *C. jejuni* detection varied between 3.8% and 13.1%, respectively, in diarrheic feces from HIV seropositive individuals<sup>5,54,73</sup>. On performing specific investigations of non-*jejuni* species, it was found that other members of the *Campylobacter* sp. genus are frequently detected in the stools of HIV-infected patients<sup>83</sup> and also that these may be associated with prolonged mild to moderate diarrhea in HIV-infected patients<sup>49</sup>. Another aggravating fact is that patients under continuous antibiotic therapy, a situation common among HIV-infected individuals, have greater tendency to carry resistant strains of all the bacterial genera mentioned above, to the main treatment and/or chemoprophylactic drugs such as trimethoprim-sulfamethoxazole, nalidixic acid, erythromycin, ampicillin and quinolones<sup>13,54,57,83</sup>. The *Mycobacterium* sp. species, especially from the *M. avium* complex (MAC), were also detected in the feces of HIV-infected patients at frequencies of around 15% but without significant association with diarrhea<sup>46,56,62,68</sup>. However, case reports show

the unequivocal involvement of non-tuberculous mycobacteria, such as *M. ulcerans*, in chronic diarrhea<sup>47</sup> and *M. avium* intracellular-caused duodenitis in these patients<sup>79</sup>.

## Virus

Viral enteritis is a well known manifestation among HIV seropositive patients, but the involvement of its etiological agents seems to vary greatly depending on the origin and age of the population assessed. In general, the percentage of detection of at least one enteric virus ranged from 6.4 to 52% in Venezuelan HIV-infected individuals<sup>42,59</sup> and from 20 to 35% in HIV seropositive populations from Argentina and the United States of America<sup>39,43</sup>. Rotavirus is one of the main causes of infantile gastroenteritis due to the greater exposure of children<sup>87</sup>. Among the HIV seropositive infantile population, the data are divergent depending on the geographical region considered with frequencies of isolation of 0%, 5% and 30% in Brazilian<sup>78</sup>, Venezuelan<sup>59</sup> and Malaysian<sup>21</sup> children, respectively. Also, in studies of adult HIV seropositive populations, rotavirus was detected in 18% of the patients in a study in England<sup>89</sup>, 13.6% in Germany<sup>1</sup>, 8.2% in Senegal<sup>35</sup> and in 2.5% of samples collected in the Northern region of Brazil<sup>33</sup> and so demonstrated considerable regional and/or seasonal variability.

Astroviruses are members of the *Astroviridae* family and are recognized as a common cause of gastroenteritis not only in infants but also among elderly and immunocompromised patients<sup>63</sup>. The main symptom of infection by astroviruses is a watery diarrhea that may be associated with fever, vomiting, anorexia and abdominal pain<sup>32</sup>. In Venezuela and Argentina, the virus was undetected<sup>42</sup> or detected at frequencies of 4%<sup>39</sup> in people with HIV, respectively, without, however, being significantly associated with diarrhea. The same was observed in the Southeast of Brazil among seropositive non-diarrheic children, but with a higher frequency of isolation (11%)<sup>78</sup>. Viruses such as picobirnavirus and calicivirus have frequently been described in diarrheic and non-diarrheic fecal samples of individuals with HIV. Picobirnaviruses have been identified in the feces of several species of vertebrates, including humans, but their associations with diarrhea in animals or among HIV seronegative individuals are not clear. Nevertheless, among HIV positive patients, infection by picobirnavirus has been significantly associated with diarrhea<sup>38,39,43</sup>, with reports that such patients have problems on clearing this infection<sup>43</sup>. Caliciviruses are considered the principal cause of viral outbreak in the world and more recently as one of the main causes of acute gastroenteritis among children. A wide variation in positivity (6% to 51%) was demonstrated involving both HIV seropositive adults and children<sup>30,42,77,89</sup> which indicates that future studies should attempt to elucidate their participation as etiological agents of diarrhea in these populations. Recent data on an adult HIV seropositive population from Northeastern São Paulo State showed a significant association between calicivirus detection and diarrhea in a case-control study<sup>40</sup>.

## Yeast

Despite of bacteria, parasites and viruses being the most important enteropathogens that cause infectious diarrhea, the importance of *Candida* sp. as a colonizer of the gastrointestinal mucosa of HIV-positive patients is unquestionable, mainly in respect to the group denominated as "non-albicans". The isolation of *C. albicans* in stools of patients from differing countries such as Senegal<sup>35,36</sup>, Cameroon<sup>81</sup>, India<sup>3</sup> and Ivory Coast<sup>88</sup> has shown a variation of 7.6% to 39.1%. In Brazil, the results of

ROSSIT *et al.*<sup>78</sup> were comparable with the highest published frequencies (35%) reported in HIV seropositive children. Indeed, these pathogens have been reported as opportunistic in the gastrointestinal tract and are considered in some situations the only etiological agent necessary for diarrheic symptoms<sup>15,27</sup> or relevant only when associated with other microorganisms<sup>14,31,53</sup>. Anyway, their importance is based on the growing incidence of isolation in oral and esophageal mucous membranes and even in the blood stream (candidemia) and their intrinsic or acquired resistance to the main available antifungal drugs<sup>78</sup>. This raises the need for additional systematic investigation on the participation of this genus on infectious diarrhea etiology among the HIV positive group.

## Parasites

Infections caused by intestinal parasites have been studied much more than any other infection in HIV seropositive individuals and AIDS patients. These infections receive special attention as the cause of diarrhea. In the context of the commonest identified parasites are *Giardia lamblia*, *Entamoeba histolytica*, *Strongyloides stercoralis*, *Ascaris lumbricoides* and *Ancylostoma duodenale*<sup>12,26,48,74</sup>. Apart from the classical protozoa, emergent species such as *Cryptosporidium parvum*, *Microsporidia*, *Cyclospora cayetanensis* and *Isospora belli* have also been frequently mentioned in the literature<sup>9,14,26,74</sup>.

A study in India identified different pathogens related to diarrhea in HIV-1 infected patients. *C. parvum* presented with the highest frequency (33%) but *G. lamblia* (13.3%), *Microsporidium* (6.7%) and *I. belli* (2.7%) were also detected. The authors also observed that the cases of chronic diarrhea were statistically associated to polyparasitism and with lower TCD4<sup>+</sup> cell counts<sup>26</sup>. In another region of the country, an investigation of the HIV seropositive adult population with acute or chronic diarrhea identified *E. histolytica* (14.9%) as the most prevalent enteroparasite followed by *Cryptosporidium* (8.5%), *S. stercoralis* (5.3%) and *G. lamblia* (4.3%)<sup>50</sup>. On the other hand, DEODHAR *et al.*<sup>25</sup> identified *Cyclospora* in 6.6% of patients with AIDS in an Indian hospital and found that half of these had infections which were mainly associated with *Cryptosporidium*. Investigations of adult African patients infected by HIV demonstrated a varied panorama of enteroparasites, both in diarrheic patients and in non-diarrheic individuals. In non-diarrheic patients, *A. lumbricoides* was the most common helminth identified (30.8%) followed by *S. stercoralis* (5.1%). In respect to the protozoa in this group, *E. histolytica* (10.3%) and *G. lamblia* (3.8%) were the commonest<sup>45</sup>. In patients with diarrhea, *Cryptosporidium*, *Microsporidium* and *E. histolytica* were the most prevalent intestinal protozoa<sup>35,64</sup>.

In Europe, an investigation of patients with HIV in South Italy also demonstrated a significant association between intestinal symptoms and TCD4<sup>+</sup> lymphocyte counts below 200 cells/mm<sup>3</sup>. In this work, among the 65 patients with diarrhea, the following protozoa were identified: *C. parvum* (21.5%), *Blastocystis hominis* (10.7%), *Microsporidium* (9.2%), *G. lamblia* (6.1%) and *I. belli* (1.5%)<sup>7</sup>. In Americas, two researches conducted in adult patients infected by HIV-1 in Peru, demonstrated that *Cryptosporidium* was the main protozoan associated with diarrhea<sup>13,34</sup>. Additionally, in Cuba, *C. parvum* was found in 13.1% of all HIV seropositive patients and a positive association was verified with less than 200 TCD4<sup>+</sup> cell count<sup>23</sup>. Apart from this enteroparasite, *G. lamblia*, *S. stercoralis* and *I. belli* were also evidenced but without this association. Interestingly, KAMINSKY *et al.*<sup>51</sup> studied HIV seropositive individuals in Honduras and did not identify any cases of giardiasis, amebiasis and cryptosporidiosis. The authors demonstrated a strong association

between eosinophilia and the presence of intestinal helminthosis caused by *Trichuris trichiura* (11%), *A. lumbricoides* (12%) and *S. stercoralis* (13.2%). On the other hand, other authors in the same country detected *C. parvum* (7.7%), *S. stercoralis* (7.7%), *C. cayetanensis* (1.9%) and *B. hominis* (1.9%) exclusively in persons infected with HIV<sup>58</sup>, and, also, no association with diarrhea was observed. In Haiti, the infection by *I. belli* (15.0%) was associated with chronic diarrhea and weight loss in patients with AIDS<sup>24</sup>. Furthermore, in United States, three earlier reports associated *C. parvum* as an important pathogen in HIV patients, and its frequency ranged from 3.8% to 10.0%<sup>28,66,84</sup>. Moreover, the prevalence of other enteric parasites among these HIV-infected attendees of the AIDS clinic was: *G. lamblia* (55%), *I. belli* (10%) and *E. histolytica* (3%)<sup>28</sup>.

In Brazil, some studies have evaluated the frequency of enteroparasites in the HIV population as well as their association with TCD4<sup>+</sup> cell counts, viral load and the presence of diarrhea. In fecal samples collected from in-patients in a hospital in Ceará State, Northeastern region of Brazil, where 49% had AIDS, a significant association was found between the presence of *C. parvum* and *Microsporidium*<sup>97</sup>. In contrast, in the same Brazilian region, in an AIDS patients' service of a university hospital in Bahia State, no association was observed for the TCD4<sup>+</sup> lymphocyte count, the viral load and the presence of intestinal parasites. However, the presence of *S. stercoralis* and *G. lamblia* was greater among individuals infected by the HIV<sup>29</sup>.

In Southeastern Brazil, where the highest number of cases (67.8%) of individuals infected by HIV and with AIDS in the country is found<sup>10</sup>, the panorama of infections and associations with the clinical characteristics of individuals infected by HIV also showed variability similar to that described above<sup>78</sup>. In the State of Rio de Janeiro, two published studies did not report any positive association between the presence of enteroparasites and diarrhea<sup>67,75</sup>. However, *Cryptosporidium* was the commonest protozoan. Additionally, MOURA *et al.*<sup>67</sup> detected the presence of other parasites in the feces of HIV/AIDS patients attended in a university hospital, including *S. stercoralis* and *G. lamblia* (15.2%), *E. histolytica* (13.1%), *A. lumbricoides* (11.1%) and *I. belli* (10.1%). In Uberlândia, State of Minas Gerais, the occurrence of infections by intestinal parasites in AIDS patients was 12%, infected by *S. stercoralis*, 7% by *I. belli*, 4% by *Cryptosporidium* sp. and 4% by helminth infections. Of these patients, those classified as chronic alcoholics were more commonly infected by *S. stercoralis* (64.3%)<sup>82</sup>. In another research in Uberaba, also in the State of Minas Gerais, patients with diarrhea and TCD4<sup>+</sup> counts of less than 200 cells/mm<sup>3</sup> presented with high frequencies of *I. belli* (10.3%) and *Cryptosporidium* (8.6%), thereby demonstrating the opportunistic profile of these infections and their relationship with the immunological state of the individual<sup>22</sup>. The authors also reported the impossibility of an association with highly active antiretroviral therapy (HAART), as only 8.5% of HIV patients positive for these coccidia received this therapy regularly, and they observed that the seasonal factor was important for justifying the presence of *Cryptosporidium*. An investigation of HIV patients in the city of São Paulo found a prevalence of 14.3% for *Cryptosporidium* sp. in patients with AIDS and with diarrhea, which was statistically significant for patients with low TCD4<sup>+</sup> counts<sup>76</sup>. Subsequently, another investigation in the same city, demonstrated a significant association between diarrhea and the presence of cryptosporidiosis, giardiasis and isosporidiasis. However, no association was identified between TCD4<sup>+</sup> counts and the detection of any specific parasite<sup>18</sup>. In the Northwestern region of São

Paulo State, three studies have been carried out on this theme; two in the adult population<sup>14,41</sup> and the other in infants<sup>78</sup>. In the studies of 2004 and 2007 no association was found between the presence of intestinal parasites and diarrhea. However, in the infant population the consumption of raw foods and severe immunosuppression were associated with this symptom. In these two studies the frequencies of *Cryptosporidium* were 9.09% and 62%, of *G. lamblia* were 4.04% and 9% and of *E. histolytica* were 0% and 1%, respectively. Recently, in the third evaluation on the same Brazilian region, HIV seropositive adults had diarrhea positively associated with *G. lamblia* in a case control study carried out during the years of 2006 and 2007<sup>41</sup>. This point to the need for a continuous surveillance of enteroparasites in this population.

### Concluding remarks

It is important to emphasize that the greatest prevalence of emergent agents is seen, without doubt, in the more advanced stages of AIDS, a phenomenon that is observed after the start of antiretroviral therapy and which greatly affects survival<sup>5,6,8,38,42</sup>. Thus, infection by HIV may be accompanied by severe cases of diarrhea thereby instigating important studies on opportunistic and saprophyte pathogens<sup>18</sup>. Still further elucidation is necessary in order to identify any association between diarrhea, so common in sufferers with this virus and the low TCD4<sup>+</sup> cell counts. Hence, the criteria utilized to establish specific treatment should be analyzed including the severity of diarrhea which depends on the immunological state of the patient and of the virulence of the pathogen involved<sup>8,9</sup>. These studies are important tools for interventions in patients with HIV/AIDS, enabling a better understanding of the etiology of diarrhea<sup>13</sup>, as well as favoring the quality of life of these patients.

Moreover, the colonization by potential pathogenic agents verified in asymptomatic HIV-seropositive individuals is high which may result from immunologic tolerance, virulence variations, or drawn-out liberation of microorganisms after the last episode of diarrhea<sup>11</sup>. This review emphasizes that although some of these studies showed no association between diarrhea and a specific enteropathogen, the HIV/AIDS population still harbor these organisms and act as carriers. Therefore, their potential role in dissemination must be further evaluated. Important questions for diarrhea controlling in the HIV seropositive population, such as the identification of other opportunistic agents, beyond subgroups, variants, and pertinent factors of virulence, remains to be answered<sup>78</sup>. To accomplish this goal, improving traditional diagnostic and/or creating new laboratory methods is crucial.

The differences on the importance of each agent may be due to local geographical differences and/or the fact that different studies evaluated distinct groups of enteric pathogens. Finally, public health measures of control and prevention must take into consideration the regional previously identified enteropathogens, especially in areas where HIV prevalence is high.

### RESUMO

#### Agentes etiológicos da diarreia em pacientes infectados pelo vírus da imunodeficiência tipo-1: revisão

Muito embora a importância do conhecimento sobre a epidemiologia dos agentes responsáveis pela diarreia infecciosa nos portadores do

vírus da imunodeficiência humana (HIV) e/ou doentes pela síndrome da imunodeficiência adquirida (SIDA), o número de artigos científicos sobre o tema é relativamente pequeno. Este artigo resume os dados já publicados sobre os enteropatógenos bacterianos, fúngicos, virais e parasitários que acometem os indivíduos soropositivos para o HIV e/ou doentes pela SIDA em diferentes países, regiões e localidades. Em geral, existe uma grande diferença nas frequências dos agentes etiológicos, devido a fatores que incluem status imunológico, localização geográfica, clima e condições socioeconômicas. Ressalta-se, que é grande a prevalência de infecção por agentes emergentes nos estágios mais avançados da SIDA. Portanto, o estabelecimento de tratamento específico depende diretamente do conhecimento desses agentes bem como dos fatores de risco associados à sua distribuição. Ademais, a colonização por patógenos em potencial nesses indivíduos é elevada o que determina o estado de portadores assintomáticos. Por fim, medidas em saúde pública relativas ao controle e prevenção da diarreia nessa população devem considerar os enteropatógenos de importância regional, especialmente em áreas onde a prevalência do HIV é elevada.

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