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CASE REPORT

INTESTINAL AND PULMONARY INFECTION BY *Cryptosporidium parvum* IN TWO PATIENTS WITH HIV/AIDS

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SUMMARY

We describe two patients with HIV/AIDS who presented pulmonary and intestinal infection caused by *Cryptosporidium parvum*, with a fatal outcome. The lack of available description of changes in clinical signs and radiographic characteristics of this disease when it is located in the extra-intestinal region causes low prevalence of early diagnosis and a subsequent lack of treatment.

KEYWORDS: HIV/AIDS; Cryptosporidiosis; Pneumonia; *Cryptosporidium parvum.*

INTRODUCTION

Cryptosporidium parvum is an obligate intracellular parasite of the Coccidia class that infects the microvilli epithelial cells of the digestive and respiratory systems[1]. This parasite is responsible for causing severe diarrhea in approximately 55% of human immunodeficiency virus/ acquired immune deficiency syndrome (HIV/AIDS) patients living in developing countries[2].

Among the 16 currently described species of *Cryptosporidium, C. parvum and C. hominis* are those that predominate in immunocompromised individuals[3].

Infection occurs after ingestion of water or food contaminated with oocysts or direct person-to-person or animal-person contact[4]. Respiratory forms of infection can happen upon inhalation of oocysts during an episode of vomiting[5,6].

Studies by LOPEZ-VELEZ *et al.*[7] and CLAVEL *et al.*[8] found that 30.2% of patients with intestinal cryptosporidiosis also carried extraintestinal infections in both the lungs and bile. The studies mentioned above reported high mortality rates amongst the patients as these cases showed dramatically lower CD4 + cell counts, ultimately reflecting a very severe degree of immunosuppression.

Therefore, we decided it to be of clinical and educational significance to report our finding of *C. parvum* (identified by molecular testing) in fecal samples and sputum from two HIV/AIDS patients.

This study was submitted and approved by the Research on Human Beings Ethics Committee of the *Marília* Medical School (FAMEMA), under the number 33677514.1.0000.5413. Figure 2 was made using the medical records of patients what are in the custody of the *Serviço de Prontuário de Paciente* (SPP) of *Marília* Medical School. Similarly, the blades for the manufacture of Figure 3 were photomicrographed in Olympus microscope BX41 model coupled to a digital video camera, Olympus, DP 25 model, DP2-BSW software, Olympus, originals of FAMEMA Parasitology and Microbiology Laboratories.

Patient history

Case 1:

On 10/06/2013, a fifty-nine-year-old female patient sought medical care at the emergency ward of the *Marilia* Clinical Hospital. The patient reported a two-month-long history of diarrhea, along with oral moniliasis associated with weight loss of 15 kg +/- over this period. She denied any fever, cough, urinary changes or emesis. The patient reported one prior medical consultation within Brazil's Specialized Care Service (SAE) with the same story and referring indication of highly active antiretroviral therapy (HAART), however, without any confirmed CD4 + result.

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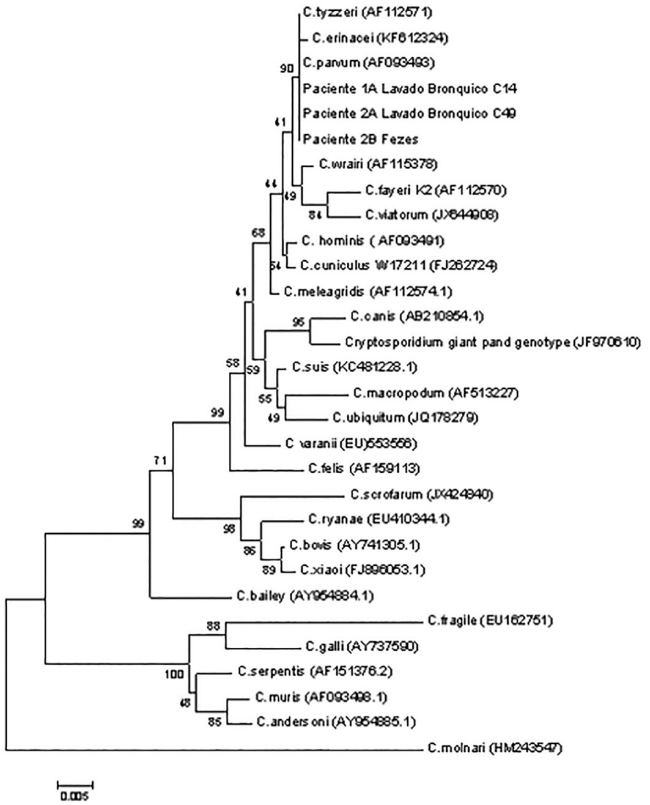

Fig. 1 - A phylogenetic tree was inferred using the neighbor-joining method[28] with 1000 bootstrap replicates, and evolutionary distances calculated using the Kimura 2-parameter[29] method. Evolutionary analyzes were performed by MEGA5 software[30].

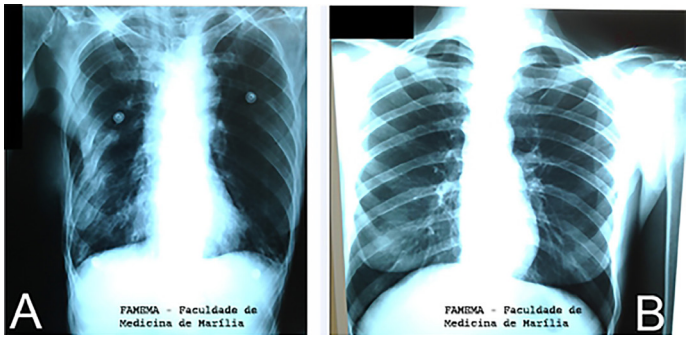

Fig. 2 - **A:** Nonspecific radiographic changes presented by Patient 1. **B:** Patient 2 without significant radiographic changes.

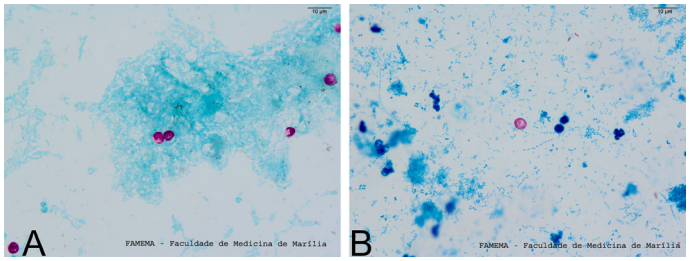

Fig. 3 - A: *Cryptosporidium* spp. oocysts in faeces stained by the Kinyoun method. **B:** *Cryptosporidium* spp. oocysts in sputum stained by the Ziehl-Neelsen method.

On the day of treatment, the patient had a physical examination that determined a poor general condition: pale; dehydrated; Heart rate: 68 bpm; Blood pressure: 80 x 50 mmhg; afebrile.

Lungs: Vesicular murmur: present with bibasilar crepitant rales.

Heart: S_1 and S_2 heart sounds normal and regular; without murmurs.

Abdomen: excavated, bowel sounds present and hypoactive, limp and painless.

Lower limb: weak pulse, with capillary refill time: 3 seconds.

At this time, supportive measures were instituted, including a broad spectrum treatment for opportunistic diseases. Laboratory tests were requested, including for detection of *Cryptosporidium* spp., which ultimately proved positive in both stool samples (method Kinyoun) and sputum (the Ziehl-Neelsen).

Case 2:

On 10/18/2013, a forty-four-year-old female patient was admitted to the Infectious Diseases Ward of *Marilia* Clinical Hospital. The patient reported experiencing diarrhea for approximately the previous three months. During this period, the patient experienced a weight loss of 10 kg, associated with an unmeasured fever and dry cough. On the day of admission, the patient presented a normal general condition: conscious, oriented, dehydrated and pale. Heart: S_1 and S_2 heart sounds normal; without murmur Heart rate: 86 bpm, Lungs: Vesicular murmur: present, no adventitious sounds (Saturation: 98%).

Supportive measures and laboratory tests were performed.

Treatment of esophageal candidiasis was initiated upon hospitalization. An Acid-Alcohol Resistant Bacilli (AARB) exam was performed with negative results, and the analysis of stool and sputum samples for *Cryptosporidium* returned positive.

Molecular testing: Stool and sputum samples from both patients were submitted to a specific detection of *Cryptosporidium* using Nested PCR method. The fragment of the 18S rRNA subunit was amplified by nested PCR by using the forward primers: SCL1F 5'-CTGGTTGATCCTGCCAGTAG-3' and reverse CPB-DIAGR 5'-AAGGTGCTGAAGGAGTAAGG-3' which amplify 1035 pb and SSU F 5'-GGAAGGGTTTTTATTGTAAGATAAAG-3' and SSUR 5'-AAGGAGTAA GGATCCACCACAA- 3' which amplify about 826 pb as previously described by COUPE *et al.*[9] and XIAO *et al.*[10] to identify of *Cryptosporidium* spp. The fragments of the secondary PCR were purified with GFX™ PCR DNA Gel Band Purification Kit (GE-UK) and directly sequenced. The nucleotide sequences obtained were analyzed and compared with those registered in GenBank, and phylogenetic analysis were used on the aligned sequences to assess relationships among sequences that allowed the identification of *Cryptosporidium parvum* in both patients.

DISCUSSION

The low number of diagnosed cases of extraintestinal cryptosporidiosis,

especially of pulmonary location, is largely due to the absence of specific clinical signs, as well as the presence of radiological abnormalities, which can be confused with other opportunistic infections that commonly affect patients with immune impairment.

In our cases, parasitological examination by Ziehl-Neelsen and Kinyoun techniques demonstrated the presence of *Cryptosporidium* spp. in the sputum and stool (Fig. 3) of two patients. Confirming the presence of the specific species, *C. parvum,* was performed by nested PCR of the 18S rRNA region in sputum and feces of patient 2, and sputum of patient 1. Sequencing of the samples was then performed and aligned with the sequences available in the GenBank database, showing similarity with the *C. parvum* species, as can be observed in Figure 1.

There is no sufficient molecular data available to support information on the prevalence of *C. parvum* in clinical cases in Brazil. ASSIS *et al.*[11] detected *Cryptosporidium parvum* in 10 HIV-positive patients in *Minas Gerais*, suggesting a possible zoonotic transmission of this species of *Cryptosporidium*. However, many studies showed that *C. hominis*, along with *C. parvum*, are the most frequent species observe in humans worldwide[12,13].

The differentiation of *Cryptosporidium* to species level depends on the identification by specifics molecular methods, which limits the availability of information on the spread of this species of *Cryptosporidium* in human clinical cases, mainly in Brazil[12]. Therefore, the epidemiological importance of reporting these cases is bolstered by the fact that the transmission of this parasite occurs from person-person[14], since certain promiscuous habits can happen primarily amongst practitioners of oro-anal intercourse, putting these individuals at specific risk of infection[12,15,16].

Although the results demonstrated the presence of *C. parvum* in stool and sputum, indicating the possibility that pulmonary infection has occurred through inhalation of oocysts during an episode of vomiting, as mentioned by some authors[6], one cannot rule out the spread by hematogenous route, since it is described as the presence of the parasite within macrophages[17].

GENTILE *et al.*[18] reported finding *Cryptosporidium* oocysts within blood vessels and studies of MARTINEZ *et al.*[19] in an *in vitro* study conducted in mice, demonstrated that *Cryptosporidium* can multiply within macrophages, resisting the action of lysosomal enzymes, making it difficult to control the infection in immunocompromised patients in case that this possibility occurred in humans.

Although radiographic changes occurred only in Patient 1, (Fig. 2), specific treatment for cryptosporidiosis was not instituted, because until then there was no effective treatment for cryptosporidiosis in HIV/AIDS patients. Several authors showed the limited effectiveness of drugs like Nitazoxanide, Macrolides, or Paromomycin for treating cryptosporidiosis in immunocompromised patients, even if HAART was associated[20,21,22,23]. Recently, promising results have been obtained by CASTELLANOS-GONZALEZ *et al.* (2013)[24] when they treated immunosuppressed mice infected with *C. parvum* using Calcium-dependent protein kinases inhibitor (CDPK1 inhibitor).

Therefore at this time, we choose to treat the most prevalent infectious diseases in these patients, and antiretroviral therapy HAART was initiated to recovery the cellular immune system[25], since CD4+ cells are decisive for the acquired immune response[26]. However, in a short space of time, there was a worsening of the general condition of Patient 1, and she progressed to death. In relation to Patient 2, as she had been clinically stable, the patient was discharged with guidelines for outpatient follow-up and established HAART. However, the patient did not return to her scheduled consultations, resulting in an interruption in medical care and, consequently, the maintenance treatment, leading to a fatal outcome.

Seeing as a pulmonary infection, it is considered a rare complication in its intestinal form[27], combined with the high mortality rate of these cases, the lack of formal description of clinical and radiographical changes seen upon extra-intestinal localization of this parasite, cause an extremely low rate of early diagnosis and untimely treatment.

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