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BRIEF COMMUNICATION

Cyclospora cayetanensis IN SPUTUM AND STOOL SAMPLES

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SUMMARY

We report the observation of acid-fast *Cyclospora cayetanensis* oocysts in a sputum sample. The patient, a 60 year-old, HIV negative man, was successfully treated for pulmonary tuberculosis during 1997. On February 1998, he was admitted to our center due to loss of weight, cough with purulent expectoration, dysphonia and a radiological picture of pulmonary fibrosis.

Bacilloscopic study of sputum (negative for acid-fast bacilli) stained with Ziehl-Neelsen technique showed large (8-10 µm) spherical, acid-fast *Cyclospora cayetanensis* oocysts. No other pathogens were isolated on cultures from this sample or from laryngeal biopsy. Serial parasitologic studies showed *C. cayetanensis* and also eggs of *Trichuris trichiura*, *Ascaris lumbricoides* and *Hymenolepis nana* and of *Entamoeba coli* cysts.

The patient lives in the outskirts of Buenos Aires in a brick-made house with potable water and works as builder of sewers. He travelled in several occasions to the rural area of province of Tucumán which has poor sanitary conditions.

C. cayetanensis is an emergent agent of diarrhea and as far as we know this is the first time the parasite is observed in respiratory samples.

KEYWORDS: *Cyclospora cayetanensis*; Cyclosporosis; Pulmonary cyclosporosis; Intestinal cyclosporosis; Epidemiology of *Cyclospora cayetanensis*.

INTRODUCTION

Cyclospora cayetanensis has been observed as the emerging cause of diarrhea in immunocompetent and immunocompromised hosts in different areas of the World^{2.5}. Routinely, the diagnosis of *Cyclospora* infection is achieved by microscopic observation of large (8-10 μ m in diameter) and spherical oocysts, partially acid-fast stained with the Kinyoun method or by autofluorescence under UV illumination, in stool samples^{2.3}.

The aim of this report is communicate the unusual presence of *Cyclospora cayetanensis* in our media as well as its presence in a respiratory specimen from a multiparasitized and tuberculous patient.

CASE REPORT

The patient was a 60 year-old man, HIV negative, admitted in our center on February 1998 due to productive cough, thoracic pain,

dysphonia and weight loss. A chest X-rays radiograph showed the presence of bilateral fibrosis.

He had a previous admission for pulmonary tuberculosis in 1997, successfully treated with Isoniazid, Rifampin and Streptomycin. At present he has negative bacilloscopy and culture for *Mycobacterium tuberculosis*. Laryngoscopic study showed paralysis of the left vocal cords without morphological changes in the corresponding biopsy.

The patient was born in the province of Santiago del Estero (a rural media in the inner part of the country) and travelled to the province of Tucumán on several occasions. He works as a sewer builder and lives with his wife and eleven children in a brick-made house with potable drinking water in the outskirts of Buenos Aires (San Fernando City).

On February 1998, in a bacilloscopic study stained with the Ziehl-Neelsen method we accidentally observed spherical acid-fast oocysts (8-10 µm in diameter) of *Cyclospora cayetanensis* (Fig. 1). This finding

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was repeated in 2 fecal samples as well as in a fresh sputum smear stained with de Kinyoun method. Also direct microscopy of sputum showed oocysts of *C. cayetanensis* (Fig. 2). Due to the lack of technical resources in our Laboratory, no other studies, as auto-fluorescence, were carried out with the clinical samples, to obtain additional confirmation.

Fecal serial parasitologic study also showed eggs of *Trichuris* trichiura, Ascaris lumbricoides, Hymenolepis nana and of Entamoeba coli cysts.

The patient was orally treated with Trimethoprim (160 mg) – sulfamethoxazole (800 mg) twice a day during 10 days for *Cyclospora* infection and with albendazole (200 mg/day) for 10 days for the other parasitic infections. Parasitologic post-treatment control in sputum and stool samples was negative for *Cyclospora* and other intestinal parasites.



Fig. 1 - Acid-fast oocysts of *Cyclospora cayetanensis* (Ziehl-Neelsen stain) in sputum sample (1,000 X).

DISCUSSION

The presence of *Cyclospora cayetanensis* has been reported only occasionally in our country, where it has been found in fecal samples of pediatric HIV positive patients with diarrhea⁶. To date, there are no published references on pulmonary infection by *Cyclospora cayetanensis*, as it has been observed with *Cryptosporidium parvum*, another acid-fast whit smaller oocysts which can be mistaken by *Cyclospora*. Nevertheless, both parasites are not definitely similar and present differences regarding autofluorescence, size, acid-fast staining pattern and epidemiological aspects^{1,3,5}.

The pathogenic activity of this parasite on pulmonary respiratory mucosa could not be confirmed by bronchial biopsy, although no other pathogens were observed or isolated from several sputum samples.



Fig. 2 - Cyclospora cayetanensis oocyst in direct microscopy of sputum (1,000 X).

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We think that, in this case, *Cyclospora* might be considered as an opportunistic infection, acquired on previous pulmonary tuberculosis at work by inhalation. Oocysts were also found in fecal samples from the patient, whom despite his multiple intestinal parasitism did not present diarrhea.

The patient showed laryngeal paralysis suspected as a localization of the previous tuberculosis. Nevertheless, the biopsy showed no granuloma or acid-fast bacilli and the cultures were negative for *Mycobacterium* and other pathogens.

The origin of the infection could be related to the travels the patient frequently made to rural areas of the Tucumán province (where the sanitary conditions are poor), probably due to consumption of contaminated food and water. In additional serial parasitologic studies, *C. cayetanensis* was found in a fecal smear from one out of the 5 sons of the patient, who lived 3 months in the rural area of Tucumán.

In the future, the presence of *C. cayetanensis* in patients coming to the same area of the outskirts of Buenos Aires or nearby zones will have to be investigated more exhaustively in our laboratory, employing more specific stain techniques for the observation of this parasite⁷.

Parasitologists should be trained for the visualization of this parasite in smear of fecal samples or other specimens stained with the Kinyoun technique and physicians should be familiarized with the clinical finding and treatment of this parasitic infection, either in immunocompetent and immunocompromised hosts.

The prevalence of *Cyclospora cayetanensis* in the population of San Fernando City has to be investigated. In the future it is also important to address the natural reservoir for this parasite in the outskirts of Buenos Aires.

RESUMO

Cyclospora cayetanensis em amostra de escarro

Comunicamos a observação de grandes oocistos (8-10 µm de diâmetro) esféricos, ácido-álcool-resistentes de *Cyclospora cayetanensis* em amostra de escarro corada com a técnica de Ziehl-Neelsen. Na amostra não foram observados nem cultivados outros agentes patogênicos.

Trata-se de um paciente do sexo masculino, 60 anos de idade, HIV (-), tratado previamente para tuberculose pulmonar (1997). Em fevereiro de 1998 apresentou-se em nosso hospital com perda de peso, tosse com expectoração purulenta, disfonia e imagens radiológicas de fibrose pulmonar.

As culturas das amostras de escarro e da biopsia de laringe foram negativas. O exame parasitológico seriado de fezes mostrou ovos de *Ascaris lumbricoides, Hymenolepis nana* e *Trichuris trichiura* e cistos de *Entamoeba coli.*

O paciente mora nos arredores de Buenos Aires numa casa de alvenaria com água potável e é construtor de fossas (cloacas). Realizou varias viagens à zona rural da província de Tucumán.

Cyclospora cayetanensis é um agente emergente de diarréia e esta é, até onde nós sabemos, a primeira observação de oocistos deste parasito em amostra de vias respiratórias.

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REFERENCES

- CLAVEL, A.; ARNAL, A.C.; SANCHEZ, E.C. et al. Respiratory cryptosporidiosis: case series and review of the literature. Infection, 24: 341-346, 1996.
- CONNOR, B.A. Cyclospora infection: a review. Ann. Acad. Med. Singapore, 26: 632-636, 1997.
- EBERHARD, M.L.; PIENIAZEK, N.J. & ARROWOOD, M.J. Laboratory diagnosis of Cyclospora infections. Arch. Path. Lab. Med., 121: 792-797, 1997.
- HENDRICKSON, D.A. & KRENZ, H.M. Reagents and stains. In: BALLOWS, A.; HUSLER, W.J.; HERMANN, K.L.; ISEMBERG, H.D. & SHADOMY, H.J. Manual of clinical Microbiology. 5.ed. Washington, American Society for Microbiology, 1991. p. 1289-1314.
- SOAVE, R.; HERWALDT, B.L. & RELMAN, D.A. Cyclospora. Infect. Dis. Clin. N. Amer., 12: 1-12, 1998.
- SORDELLI, N.; SAREDI, N.; FALLO, A. *et al.* Diarrea por *Cyclospora* en pacientes pediátricos HIV (+). In: CONGRESO LATINOAMERICANO DE PARASITOLOGÍA, La Habana, Cuba, 1997. Resúmenes. p. 38.
- VISVESVARA, G.S.; MOURA, H.; KOVACS-NACE, E.; WALLACE, S. & EBERHARD, M.L. - Uniform staining of *Cyclospora* oocysts in fecal smears by a modified safranin technique with microwave heating. J. clin. Microbiol., 35: 730-733, 1997.

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