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Brief report

Two cases of zoonotic cryptosporidiosis in Spain by the unusual species *Cryptosporidium ubiquitum* and *Cryptosporidium felis*

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ABSTRACT

Introduction: Two cases of infection by zoonotic transmission of unusual species of *Cryptosporidium* were detected in 2010–2011 in Spain (León and Zaragoza).
Materials and methods: Cryptosporidium spp. was detected by microscopic examination of modified Ziehl–Neelsen stained fecal smears. PCR-RFLP of the *SSU*rDNA gene and sequencing of the amplified fragment confirmed the species.
Results: C. ubiquitum and *C. felis* were identified in samples from an immunocompetent child and from a HIV-positive adult, respectively.
Conclusions: This is the first report of human infection by *C. ubiquitum (cervine)* and autochthonous *C. felis*, identified in Spain.

Dos casos de criptosporidiosis zoonótica en España por las especies inusuales *Cryptosporidium ubiquitum* y *Cryptosporidium felis*

Palabras clave:
Cryptosporidium ubiquitum
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RESUMEN

Introducción: Se describen dos casos de infección por especies inusuales de *Cryptosporidium* de transmisión zoonótica detectados en España (León y Zaragoza) en 2010 y 2011.
Material y métodos: Cryptosporidium se detectó por tinción Ziehl-Neelsen modificada de la concentración de heces Las especies se determinaron por PCR-RFLP del gen *SSU*rDNA y se confirmaron por secuenciación del fragmento amplificado.
Resultados: C.ubiquitum y *C. felis* fueron identificados en muestras procedentes de un niño inmunocompetente y un adulto VIH-positivo respectivamente.
Conclusiones: Este estudio es la primera comunicación de infecciones humanas por *C. ubiquitum (cervine)* y de *Cryptosporidium felis* de origen autóctono identificados en España.

Introduction

Cryptosporidium spp. (Protozoa, Apicomplexa) is a protozoa associated with gastrointestinal disorders in humans and animals. It is a worldwide distributed parasite which has many hosts, including humans and domestic animals. It has been found in both immunocompetent and immunosuppressed patients.

Cryptosporidium hominis and *Cryptosporidium parvum* are responsible for 90% of human cryptosporidiosis in the world. *C. parvum* are relevant in humans and animals. However, *Cryptosporidium meleagridis*, *Cryptosporidium cuniculus*, *Cryptosporidium muris*, *Cryptosporidium canis*, *Cryptosporidium felis*, *Cryptosporidium suis*, *Cryptosporidium andersoni* and *Cryptosporidium ubiquitum* (*Cervine* genotype) can also infect humans, especially children and immunosuppressed patients.[1–4] *Cervine* genotype, later called *C. ubiquitum* because of the wide range of hosts and geographical areas, was found in several species of mammals (domestic and wild).[1] As far as we know, *C. ubiquitum* has been described in a few cases in human patients worldwide. It was first reported by Ong et al. in Canada in 2002.[5] It was later confirmed by several studies that *C. ubiquitum* can infect humans.

The cat is the main host of the *C. felis* genotype. It was first found in domestic cat in 1979 by Iseki.[6] Later it was also found

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in cattle and then in humans.[7] The first report of *C. felis* in humans was published by Pieniazek et al. in 1999.[8] They described three cases of *C. felis* in immunosuppressed patients. Later several studies confirmed that this genotype can infect both immunocompetent and immunosuppressed patients. *C. felis* was first described in humans in Spain by Llorente et al. in 2006 in an adopted immunocompetent 4-year-old boy who acquired the infection in Calcutta (India).[9]

The aim of the present article is to report two cases of zoonotic transmission of non-*parvum Cryptosporidium* which have occurred in recent years in Spain.

Methods

Stool samples were collected from the patients in León (Spain) in May 2010 and in Zaragoza (Spain), in July 2011. Both samples were sent to the Parasitology Laboratory of the Faculty of Medicine, University of Zaragoza, Spain.

In both cases the stool samples were concentrated with formalin–ethyl acetate. Smears from the two concentrated samples were stained with a modified Ziehl–Neelsen stain and examined by means of microscopy to detect *Cryptosporidium* oocysts. DNA was extracted using a DNA stool kit (IBIAN® DNA Stool Kit) and following the manufacturer's instructions. The samples were stored at −20°C until their processing.

A fragment of the 18S rRNA subunit (SSU rRNA) was amplified by nested PCR following previously described protocols.[10] In order to identify the species of *Cryptosporidium*, PCR restriction fragment length polymorphism (PCR-RFLP) analysis was performed with the products of the secondary PCR, which were digested with *Ssp*I and *Vsp*I enzymes at 37 °C overnight.

Additionally, PCR products of the 18S rRNA gene fragments were purified with GFX™ PCR DNA Gel Band Purification Kit and direct sequenced. The nucleotide sequences obtained were analyzed and compared with those registered in GenBank using Chromas and BioEdit.

Results

In the first case, the patient was a 6-year-old boy from a rural area who came to the University Health Care Complex of León with an abdominal pain. Although the child lived in the urban area of Leon, he spent a lot of time in the village with his grandparents who bred sheep, and contact with these animals was confirmed by the child's family.

The RFLP analysis of an amplicon of 826–864-bp, showed a band profile consistent with *C. ubiquitum* patterns: 454, 384-bp with *Ssp*I digestion and 461, 169, 115-bp with *Vsp*I digestion, and the sequence showed 99% similarity with the fragment of the various *C. ubiquitum* sequences (accession no. HM209375.1, HQ822139.1, among others).

The second patient was a 49-year-old, HIV-positive man, also from rural area, who came to a hospital in Zaragoza with diarrhea of 1-month onset, weight loss, dysphagia, sporadic fever, and generally feeling unwell. He was diagnosed with esophageal candidiasis. *Cryptosporidium* spp. and *Blastocystis hominis* were identified in this patient's fecal sample.

In this case, the RFLP analysis of the expected amplicon showed a band profile consistent with *C. felis* patterns: 426, 390-bp with *Ssp*I digestion and 476, 182, 104-bp with *Vsp*I digestion. The sequence of this fragment has maximum similarity with various *C. felis* sequences (95%, accession numbers AF356786.1, GU944848.1, among others).

The sequences of *C. ubiquitum* and *C. felis* identified in this work were registered in GenBank under accession numbers JN642225 and JQ312664, respectively.

Discussion

There are a few published studies characterizing human cryptosporidiosis in Spain. In them, molecular studies showed that there were 4 species which are the most important in human epidemiology: *C. parvum* and *C. hominis* are the most frequently found, followed by *C. meleagridis*, and *C. felis* is the least frequent.[3,11]

Publications up until now show that *C. ubiquitum* has been found in humans only in: New Zealand, Slovenia, Wisconsin, Ontario, Canada, British Columbia, Ohio and the United Kingdom.[1] On the other hand, *C. felis* infection in humans has been reported in several countries, including one case in Spain in a child adopted from an Indian orphanage.[9] As far as we know, and unlike the previous case, this report is the first autochthonous case of *C. felis* in Spain. The transmission route for the unusual *Cryptosporidium* species to humans is unclear, but *C. ubiquitum* has been previously found in lambs in Galicia (NW Spain), a geographical area near León,[12] where 5 isolates from 2 farms were identical to the *Cryptosporidium* cervine genotype 1 sequence AF442484. In this study, the sequence of PCR product of the SSU rRNA locus showed 99% identity with GenBank AF442484 of cervine genotype 1 with a single nucleotide transition at position 766 (nucleotide A was substituted for nucleotide G). In this case, the close contact between the child and animals could favor zoonotic transmission, even in the case of an immunocompetent host. Furthermore, after 15 days without contact with sheep the symptomatology of the child improved, no signs of *Cryptosporidium* were observed either in the parasitological study of the fecal samples or in the molecular study. Some authors have suggested that *C. ubiquitum* could emerge as an important human pathogen, but, until now, it has been reported only occasionally in humans. The lack of host specificity of *C. ubiquitum* and the habitat-sharing of its hosts probably contribute to it being widespread.[1] In the case of *C. felis*, the sequence obtained had only 95% homology with the one previously published, which was similar to the AF112575.1 sequence. This difference may be justified by the geographical distance between the two isolates. On the other hand, contamination with *C. felis* might favor patient coinfection with HIV and the rural place of residence. In spite of the fact that there are many of cases of cryptosporidiosis described in animals, there are only a few cases described in humans in Spain, and the *Cryptosporidium* species are not always determined, so there might be more undiagnosed cases of zoonotic cryptosporidiosis. This study shows that the zoonotic transmission cases occurred in Spain, even though its range is unknown. Further epidemiological studies on the transmission of *Cryptosporidium* species are required for a greater understanding of epidemiological aspects of this parasite and its public health risk.

Conflict of interest

The authors have no conflicts of interests to declare.

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