

## Case reports

# *Cryptosporidium meleagridis* infection: the first report in Poland of its occurrence in an HIV-positive woman

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**ABSTRACT.** *Cryptosporidium* is an opportunistic protozoan parasite that can cause severe diarrhoea in immunocompromised patients. The transmission of this pathogen in humans and animals is not fully understood. *C. meleagridis*, originally described in birds, is the only *Cryptosporidium* species known to naturally infect mammalian and avian species. This study documents the first detection of *C. meleagridis* in an HIV-infected woman in Poland.

**Key words:** *Cryptosporidium meleagridis*, HIV, AIDS, opportunistic parasites, immunocompromised patients

## Introduction

*Cryptosporidium* is a protozoan parasite which can infect a wide range of vertebrates, including humans. Infections are transmitted by the faecal-oral route through contaminated food or drinking water. Clinical manifestations are related to the immune status of the host. In immunocompetent persons, cryptosporidiosis is self-limiting, but immunocompromised patients, particularly HIV-infected individuals with low CD4+ T-cell counts (<180 cells/ $\mu$ l), can experience severe diarrhoea, which can be life threatening [1]. *C. parvum* and *C. hominis* are the most frequently reported species in human infections, but in the past few years, zoonotic *Cryptosporidium* species and genotypes, such as *C. meleagridis*, *C. felis*, *C. canis*, *C. muris*, and *C. suis*, have been detected [2,3].

*C. meleagridis*, originally described in birds, is the only *Cryptosporidium* species known to

naturally infect avian and mammalian species, as well as humans. The prevalence of *C. meleagridis* in immunocompromised individuals is estimated to be 1% of all infections in the UK and 12.6% of HIV-infected persons in Peru [3–5]. This study reports the first case of *C. meleagridis* in an HIV-infected patient in Poland.

## Case report

A 39-year-old woman living in contact with turkeys and other poultry in a small village in the Lower Silesian region had suffered from watery diarrhoea for eight months before admission to the hospital. Despite outpatient medical care, her condition worsened and her body weight fell by 20 kg during that time: her BMI was 10.17. On admission to hospital, extreme wasting, dehydration, weakness, low-grade fever, oral and oesophageal candidiasis, and pancytopenia were found.

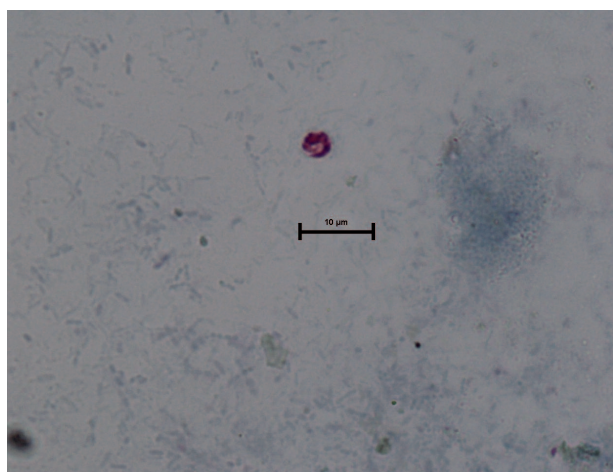


Fig. 1. *Cryptosporidium meleagridis*; oocysts stained with modified Ziehl-Neelsen method ( $\times 1000$ ) scale bar indicates

The differential diagnosis included lymphoma, leukaemia, and hypopituitarism, all of which were excluded. The patient revealed that her husband, who had been a drug user, had died of AIDS fourteen years earlier. Based on the observed symptoms and this knowledge, a test for HIV was performed, which indicated she was infected with HIV-1 and her CD4+ T-cell count was 15 cells/ $\mu$ l. Antiretroviral therapy with two nucleoside reverse transcriptase inhibitors and protease inhibitor was initiated. The patient also received symptomatic treatment. Despite this, progression of all symptoms was observed, leading to death within eight weeks.

A stool specimen was examined for intestinal parasites using conventional microscopy techniques and molecular methods. Faecal smears were prepared and stained using a modified Ziehl-Neelsen technique and examined by light microscopy at  $1000\times$  magnification.

DNA was isolated from a stool preserved in 70% alcohol using a Genomic Mini Kit (A&A Biotechnology, Poland) according to the manufacturer's recommendations. The following genome fragments were amplified: part of the gene that encodes the variable region of a small subunit of the ribosomal RNA (SSU rRNA) using the primers CPBDIAGF/CPBDIAGR, and two parts of the gene that encodes the C-terminal and N-terminal portions of the *Cryptosporidium* oocyst wall protein (COWP) using the primers CRY12/CRY14 and CRY9/CRY15, respectively [6–8]. The PCR reaction conditions were different for amplifying the particular products. The PCR products were electrophoresed on 1.5% agarose (Sigma, USA) and visualized following ethidium bromide staining.

*Cryptosporidium* was detected by both microscopic (Fig. 1) and molecular methods. Comparison of the DNA sequences of the products with data from GenBank showed that the isolate was *C. meleagridis*. The sequence of the analysed fragment of SSU rRNA as well as the sequence of the fragment of the gene that encodes the N-terminal portion of the COWP protein were in 100% agreement with similar sequences of *C. meleagridis* already deposited in GenBank. The sequence of the second fragment of the COWP gene (of the two investigated) was 95% identical to *C. parvum*, *C. hominis*, and *C. wrairi*. Analysis of the data from GenBank showed that the sequence of the *C. meleagridis* COWP gene had not previously been deposited. The obtained sequences have been deposited in GenBank under accession numbers EU284595 and EU310392.

## Discussion

Any deficiency in the human immune system enables parasites to cross the barriers to the host, resulting in the possibility of infection by parasites that usually infest animals. *C. meleagridis* is the third most common species causing human infections, behind *C. parvum* and *C. hominis* [9]. The first case of human cryptosporidiosis caused by *C. meleagridis* in Poland was identified in an immunocompromised four-year-old boy with X-linked hyper-IgM syndrome type 1 (XHIM) [10]. However, the patient described in the present report had lived for many years in a rural district where poultry, turkeys, and other domestic animals were available. Infection was probably acquired by contact with the animals, which was made possible by the advanced deficiency of the immune system caused by the HIV infection. Advanced HIV infection was also the reason for the severe course of cryptosporidiosis, leading to death.

Other studies have confirmed the zoonotic route of infection, particularly in people who have close contact with animals, for example in rural areas, and suggests that cross-species transmission of *C. meleagridis* between birds or other animals and humans is possible [11,12]. The development of numerous molecular techniques has made it possible to detect and differentiate *Cryptosporidium* species, genotypes and subtypes, which can play an important role in establishing the sources of infection with *C. meleagridis* in humans. This is particularly significant as some reports suggest that

zoonotic strains can cause more severe infections in humans, especially in immunocompromised patients, than strains found only in humans [12,13].

## Conclusions

Opportunistic cryptosporidiosis should be suspected in any patient with immune deficiency caused by HIV, who may be suffering from diarrhoea. Epidemiological data can be helpful in the proper diagnosis, but infection with unusual etiological pathogens characteristic of animals should be considered because of their changing epidemiological pattern. However, a precise diagnosis of cryptosporidiosis requires confirmation by molecular techniques.

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